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## MASTER OF SCIENCE

### Leptin induces a novel form of long-term potentiation at the direct cortical input to hippocampal CA1 synapses

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Xiao Luo

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**LEPTIN INDUCES A NOVEL  
FORM OF LONG-TERM  
POTENTIATION AT THE DIRECT  
CORTICAL INPUT TO  
HIPPOCAMPAL CA1 SYNAPSES**

**By Xiao Luo**

A thesis submitted to the University of Dundee for the Degree of Master of Science,  
November 2013

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# Declaration

I declare that I am the author of the thesis. All references cited have been consulted by me unless otherwise stated. The work mentioned in the thesis has been performed by myself, and it has not been previously accepted for a higher degree.

Signature:

Xiao Luo



# Summary

Leptin is a protein hormone that primarily regulates feeding behaviour and energy expenditure in the central nervous system. Recent studies have discovered a role for leptin in modulating hippocampal synaptic plasticity at hippocampal CA1 synapses *via* activating postsynaptic NMDA receptors. In particular, leptin induces a long-term (P5-8) or transient (P11-18) depression of synaptic transmission that depends on GluN2B subunit-containing NMDA receptors in juvenile hippocampus, whereas leptin evokes long-term potentiation that depends on GluN2A subunit-containing NMDA receptors in the adult hippocampus. Moreover, NMDA receptor subunits are heterogeneously distributed along the apical dendrites of CA1 pyramidal neurons, such that GluN2B-lacking NMDA receptors selectively concentrate at distal dendritic region of pyramidal cells where they receive direct cortical input (perforant path) from the entorhinal cortex. Indeed, mounting evidence demonstrates that the direct cortical input is of importance to spatial learning and memory. Here we show that application of leptin induced a novel form of long-term potentiation (LTP) of excitatory synaptic transmission at perforant path (PP) to CA1 synapses with normal inhibitory synaptic transmission in juvenile hippocampus. The LTP evoked by leptin exhibited concentration dependence, as treatment with a low concentration of leptin (1nM) had no effect on excitatory synaptic transmission, whereas perfusion of high concentrations of leptin (25-100nM) induced LTP of excitatory synaptic transmission that increased to ~20%. Furthermore, leptin-driven LTP had a postsynaptic locus of expression and depended on the activation of GluN2B-subunit containing NMDA receptors. In addition, the leptin-driven LTP shared similar mechanism with classical activity-dependent LTP at PP-CA1 synapses, as both process required the activation of GluN2B -containing NMDA receptors and partially occluded each other. Our results indicate that leptin plays a pivotal role in modulating NMDA receptor dependent synaptic plasticity at PP-CA1 synapses in juvenile hippocampus.

## Abbreviations

ACSF	artificial cerebral spinal fluid
AD	Alzheimer's disease
AgRP	agouti-related peptide
Akt	Protein Kinase B
AMPA	$\alpha$ - Amino- 3- hydroxy- 5- methyl- 4- isoxazolepropionic acid
AMPK	Adenosine 5'-monophosphate-activated protein kinase
ARC	arcuate nucleus
CB1	cannabinoid receptor type 1
CNS	central nervous system
DA	dopamine
DMH	dorsomedial hypothalamus
EC	entorhinal cortex
EPSC	excitatory postsynaptic current
ERK	extracellular signal-regulated kinase
fEPSP	field excitatory postsynaptic potentials
GABA	gamma-aminobutyric acid
GSK3 $\beta$	Glycogen synthase kinase 3 beta
HFS	high frequency stimulation
JAK	The Janus kinase
Kainite	2- carboxy- 3- carboxymethyl- 4- isopropenylpyrrolidine
LTD	long-term depression
LTP	long-term potentiation
MAPK	Mitogen-activated protein kinases
mGluR	metabotropic glutamate receptors
NE	Noradrenaline
NFT	neurofibrillary tangles
NMDA	N- methyl- D- aspartate
NPY	neuropeptide Y
ObR	leptin receptor
pIPSP	population inhibitory post-synaptic potentials
PI3K/Akt	phosphoinositide 3-kinase
POMC	pro- opiomelanocortin
PP	Perforant Path
PPR	paired-pulse facilitation ratio
PTEN	Phosphatase and tensin homolog
p38	mitogen-activated protein kinases
STAT	signal transducers and activators of transcription
SC	Schaffer Collateral commissural pathway
SIRT1	silent mating type information regulation 2 homolog 1
SLM	stratum lacunosum moleculare
SR	stratum radiatum
STP	short term potentiation
VGCC	voltage-gated calcium channel
VMH	ventromedial hypothalamus

$\alpha$ - MSH  
5-HT

$\alpha$ - melanocyte- stimulating hormone  
5-hydroxytryptamine

# **Chapter One**

## **Introduction**

### ***1.1: Leptin signalling and physiological effects in the hypothalamus***

Leptin is a 167 amino acid protein encoded by the *ob* gene and expressed primarily in white adipose tissue. Since the discovery of leptin in 1994, it has been well known as a hormone that restrains feeding behaviour and controls body weight, thus it is considered as a potential therapy for metabolic -related diseases (Zhang et al., 1994).

The first step in leptin signalling is the binding of leptin with various leptin receptor (ObR) isoforms, which belong to the class I cytokine receptor family (Tartaglia, 1997, Myers, 2004). Alternative splicing of *db* gene transcript gives rise to six receptor isoforms (ObRa-ObRf; (Lee et al., 1996), which can be divided into three different types: short form, long form and secreted ObR (Fruhbeck, 2006). In particular, the long form, ObRb is the only isoform that is able to activate intracellular signal pathways via its long intracellular domain (Tartaglia et al., 1995).

ObRb is abundantly expressed in the central nervous system including high levels of expression in the ventromedial hypothalamus (VMH), arcuate nucleus (ARC) and dorsomedial hypothalamus (DMH); (Schwartz et al., 1996b, Couce et al., 1997). Binding of leptin with ObRb triggers many downstream signal pathways such as JAK/STAT, ERK/MAPK, PI3K/Akt and AMPK/SIRT1 (Ghilardi et al., 1996, Bjorbaek et al., 1997, Banks et al., 2000, Gomez-Pinilla and Ying, 2010).

Circulating leptin has to cross the brain-blood barrier *via* a receptor mediated transport system (Banks et al., 1996) or enter the cerebrospinal fluid (Schwartz et al., 1996a) to reach its main targets in the central nervous system (CNS)-the hypothalamus. In the hypothalamus, leptin regulates food intake and energy homeostasis by activating two types of neurons: the neuropeptide Y (NPY) and agouti-related peptide (AgRP) expressing neurons and pro-opiomelanocortin (POMC) expressing neurons (Elmquist et al., 1998, Baskin et al., 1999, Elmquist et al., 1999). Activated ObRb receptors in POMC neurons depolarizes the membrane and enhances the biosynthesis of  $\alpha$ -melanocyte- stimulating hormone ( $\alpha$ - MSH), which activates melanocortin receptors expressed on the membrane of the second order neurons and eventually results in reduced appetite and increased energy expenditure (Huszar et al., 1997, Marsh et al., 1999, Chen et al., 2000). In NPY/AgRP expressing neurons, leptin activates ATP-sensitive  $K^+$  channels to attenuate the action potential firing frequency and ultimately reduce food intake (Spanswick et al., 1997, Takahashi and Cone, 2005). In contrast,

leptin inhibits hippocampal neurons by activating large conductance  $\text{Ca}^{2+}$  activated  $\text{K}^{+}$  channels but not ATP- sensitive  $\text{K}^{+}$  channels (Shanley et al., 2002).

### ***1.2: The role of glutamate receptors in basal excitatory synaptic transmission and synaptic plasticity in the hippocampus***

Ligand-gated glutamate receptors can be generally divided into ionotropic receptors that serve as channels to mediate rapid ion flow across the membrane, and metabotropic receptors (mGluRs), which are coupled with intracellular G proteins and activate intracellular signal pathways. Ionotropic receptors can be further catalogued into  $\alpha$ -Amino- 3- hydroxy- 5- methyl- 4- isoxazolepropionic acid (AMPA) receptors, N-methyl- D- aspartate (NMDA) receptors, and 2- carboxy- 3- carboxymethyl- 4- isopropenylpyrrolidine (kainate) receptors. Native ionotropic glutamate receptors are likely tetramers comprising at least two types of the subunits. Four isoforms of AMPA receptors have been discovered: GluA1-4, and there are three types of NMDA receptor subunits: GluN1, GluN2A-D, GluN3A-B. In rat hippocampus, the most common form of NMDA receptor is composed of two GluN1 and two NR2 (A-D) subunits (Wenzel et al., 1997).

In the hippocampus, the excitatory synaptic transmission is mediated by glutamate receptors, in particular AMPA receptors (Collingridge et al., 1983). Presynaptically released glutamate binds to postsynaptic AMPA receptors, allowing the transmembrane flow of  $\text{Na}^{+}$  ions that result in the excitatory postsynaptic potential. However, NMDA receptors play a minor role in basal synaptic transmission because the channel is blocked by  $\text{Mg}^{2+}$  ions. When glutamate binds and the postsynaptic membrane is depolarized enough to remove  $\text{Mg}^{2+}$  that blocks the channel, the channel opens and  $\text{Ca}^{2+}$  entry through the pore triggers various signalling pathways that eventually lead to the alternation in synaptic strength (Collingridge et al., 1988). In particular, long-term potentiation (LTP) and long-term depression (LTD) refer to the long lasting enhancement or reduction in excitatory synaptic transmission between neurons. The concept of LTP, originally described as long lasting potentiation (LLP) can be traced back to the work of Anderson and co-workers in the mid/late 1960's (Andersen et al., 1966, Anderson and Lomo, 1966, Bliss and Lomo, 1973). In 1973, Bliss and Lomo reported that a brief period of high frequency stimulation resulted in robust strengthening of perforant path to dentate gyrus synaptic transmission in rabbit

hippocampus, which is accompanied by increased likelihood of action potential firing (Bliss and Gardner-Medwin, 1973, Bliss and Lomo, 1973). In this pioneering paper, Bliss proposed that LTP may be a candidate mechanism of memory, as LLP could last for hours, which enables long-term storage of information from entorhinal cortex. Later, Bliss and Collingridge developed the concept of NMDA receptor-dependent LTP as a molecular substrate for learning and memory (Bliss and Collingridge, 1993). In a review published in 2003, Morris summarized psychological, anatomical and neurochemical methods for interfering with the induction of LTP, which again emphasized the essential role of LTP in learning and memory (Morris, 2003).

Heterosynaptic LTD was first observed by Levy and Steward in 1973 following high frequency stimulation of the ipsilateral perforant path (Levy and Steward, 1979). On the other hand, sustained low frequency stimulation led to long-lasting depression of synaptic transmission (homosynaptic LTD), which was also initially discovered in the hippocampus (Dudek and Bear, 1992). In contrast to LTP, LTD was considered as a mechanism involved in forgetting: the information stored in synapses previously by LTP could be erased by LTD in preparation for novel stimulus (Bear and Abraham, 1996). In addition, metabotropic glutamate receptors are also involved in hippocampal synaptic plasticity. Prolonged trains of paired pulses or antagonizing of the adenosine inhibition of glutamate release induces LTD of excitatory synaptic transmission that is independent of NMDA receptors (Kemp and Bashir, 1997a, b). This type of LTD requires the activation of group I mGluR, and subsequent local mRNA translation (Merlin et al., 1998).

### ***1.3: Leptin regulates hippocampal synaptic plasticity in an NMDA receptor-dependent manner***

The hippocampus is well known to play a pivotal role in diverse memory-associated behaviours. Since leptin receptor protein and mRNA were found abundantly expressed in the dentate gyrus (DG) and CA1 regions of the hippocampus (Huang et al., 1996, Scott et al., 2009), it is possible that leptin participates in the regulation of hippocampal-associated learning and memory. Indeed, leptin-insensitive rodents (*db/db* mice) exhibit impaired spatial memory in the Morris-water maze test (Li et al., 2002). Moreover, direct administration of leptin into the hippocampus facilitates the

behavioural performance in emotion and spatial memory related tasks (Farr et al., 2006, Oomura et al., 2006).

LTP and LTD are considered the two essential forms of activity-dependent synaptic plasticity that underline hippocampal learning and memory (Heynen et al., 1996). Accumulating data has shown that the ability of leptin to regulate hippocampal synaptic plasticity requires the activation of NMDA receptors. The impairment of LTP *in vitro* parallels the deficiency of memory-associated tasks *in vivo* in rodents with leptin receptor mutations (*db/db* mice; (Li et al., 2002). Moreover, application of leptin to acute hippocampal slice results in the conversion of short term potentiation (STP) into LTP (Shanley et al., 2001). Leptin also facilitates or suppresses the induction of NMDA receptor dependent LTP in a  $\text{Ca}^{2+}$ -dependent manner (Oomura et al., 2006). Recent studies indicate that leptin can depotentiate (or reverse) established LTP (Moult et al., 2009). In addition, under conditions of enhanced excitability, leptin treatment induces a NMDA receptor dependent *de novo* LTD at hippocampal CA1 synapses in juvenile rat hippocampus (Durakoglul et al., 2005).

Growing evidence indicates that leptin regulates AMPA receptor trafficking: the exocytosis and endocytosis of AMPA receptors mediated by various signal cascades. The insertion of intracellular AMPA receptors into the postsynaptic membrane is pivotal for hippocampal LTP (Collingridge et al., 2004). AMPA receptor trafficking to synapses requires the activation of phosphoinositide 3-kinase (PI 3-Kinase; (Man et al., 2003). PI 3-Kinase facilitates the phosphorylation of  $\text{PtdIns}(4,5)\text{P}_2$  to generate  $\text{PtdIns}(4,5)\text{P}_3$  (Cantley, 2002), which is necessary for promoting the surface expression of GluA1 receptors. Whereas the lipid phosphatase PTEN dephosphorylates  $\text{PtdIns}(4,5)\text{P}_3$ , inhibiting the PI 3-Kinase signal pathway (Machama and Dixon, 1998). Recent studies indicates that leptin preferentially increases the surface expression of GluA1 and the synaptic density of GluA2-lacking AMPA receptors in adult hippocampus *via* phosphorylation of PTEN, which in turn increases  $\text{PtdIns}(4,5)\text{P}_3$  levels. Moreover, this effect of leptin mimicked and occluded the leptin-driven increase of excitatory synaptic strength in the hippocampus (Moult et al., 2010). Conversely, the ability of leptin to reverse established LTP is accompanied by a decrease in rectification properties of synaptic AMPA receptors, indicating the involvement of altered AMPA receptor trafficking. This effect of leptin was mimicked by the GluA2-lacking AMPA receptor inhibitor philanthotoxin, indicating that endocytosis of GluA2-lacking AMPA



receptor is a potential mechanism responsible for leptin driven depotentiation (Moult et al., 2009).

Several lines of evidence have shown that leptin-induced synaptic plasticity exhibits NMDA receptor subunits preference. In *Xenopus* oocytes expressing recombinant NMDA receptors, application of leptin facilitates NMDA evoked currents in GluN1/GluN2A containing NMDA receptors and ObRbs co-expressing oocytes but not when GluN1/GluN2A containing NMDA receptors are expressed alone (Shanley et al., 2001), indicating that leptin receptor activation is required for modulation of NMDA receptor function. Treatment of cultured hippocampal neurons with leptin results in rapid enhancement of the motility and density of dendritic filopodia and subsequent increase in the number of dendritic synapses (O'Malley et al, 2007). Such effects of leptin require the activation of GluN2A-containing NMDA receptors and the downstream MAPK signal pathway (O'Malley et al., 2007). Furthermore, NR2 subunits in central nervous system undergo switching in postnatal development such that the predominance of GluN2B component at early developmental stages is gradually replaced by GluN2A (Monyer et al., 1994, Sans et al., 2000). Moult and Harvey 2011 showed that leptin-induced synaptic plasticity in rat hippocampal slices depends on distinct NMDA receptor subunits at different developmental stages and such modulation by leptin parallels the developmental switching of NMDA receptor subunits composition.

#### ***1.4: The direct cortical input to hippocampal CA1 region***

There are two main inputs that form synapses onto CA1 neurons: the Schaffer Collateral commissural pathway (SC), which is part of the tri-synaptic pathway within the hippocampus that relay the information from the entorhinal cortex (EC) layer II, and Perforant Path (PP, or temporoammonic pathway), which represents the direct projection from EC layer III neurons that terminates on distal dendritic region of CA1 pyramidal neurons. Mounting evidence indicates that the direct cortical input to hippocampal CA1 region is of equal importance as the SC pathway in learning and memory-associated behaviour. Indeed, pharmacological disruption of rat PP-CA1 input causes impairments in working memory and spatial memory tasks (Vago and Kesner, 2008). PP lesioned animals show normal performance in short-term spatial memory task, but exhibit a deficit in the consolidation of long-term spatial memory (Remondes and

Schuman, 2004). Moreover, a particular group of neurons in the hippocampus and para-hippocampal areas, which are called “place cells”, display increased firing rate when the animal is exploring a novel environment. The “place field” represents the circular environment zones in which higher firing rate of neurons are observed *in vivo* (O'Keefe and Gaffan, 1971). Rats with ablation of SC fibers shows normal place field formation and stabilization but attenuated peak firing rate and relatively larger place fields (Brun et al., 2002). Similarly, DG lesion has little impact on CA1 place fields (McNaughton et al., 1989). These data indicate that place cell firing pattern can be generated by PP input in CA1 independent of tri-synaptic pathway.

In contrast to schaffer collateral inputs to CA1, distinct characteristics have been discovered at PP input to CA1 *in vitro*. Stimulation of *stratum lacunosum moleculare* (SLM;-where PP form synapses onto CA1 neurons) triggers both fast GABA<sub>A</sub> ( $\gamma$ -aminobutyric acid) and slow GABA<sub>B</sub> feed-forward inhibition in *stratum radiatum* (SR), which is mediated by PP-activated GABAergic interneurons that form synapses on proximal dendrites of pyramidal cells (Andersen et al., 1969, Empson and Heinemann, 1995). A recent study shows that the field excitatory postsynaptic potentials at PP-CA1 synapses recorded *in vivo* have longer onset latencies and shorter time-to-peak than SC-CA1 synapses (Aksoy-Aksel and Manahan-Vaughan, 2013). The PP input also receives feedback inhibition from O-LM cells which are involved in theta oscillations *in vivo* (Gloveli et al., 2005).

*SLM* and *SR* regions have notable differences in the distribution of neuromodulator receptors and ion channels. Higher densities of dopamine (DA) receptor D1, D2, serotonin (5-HT),  $\alpha$ -adrenergic and nicotinic receptors are expressed in the *SLM* region (Björklund et al., 1987, Goldsmith and Joyce, 1994). HCN1 hyperpolarization-activated cation channels (Magee, 1998, Lorincz et al., 2002) and metabotropic glutamate receptors mGluR2 (Neki et al., 1996) are also expressed more abundantly in distal dendritic region of CA1 pyramidal cells. In contrast, muscarinic receptors, CB<sub>1</sub> receptors and the GluRD subunits of AMPA receptors are more concentrated in the *SR* region (Baude et al., 1995, Xu et al., 2010). In accordance with this distribution of receptors, DA and 5-HT strongly suppress the excitatory synaptic transmission at PP-CA1 synapses by 30-40%, but show little effect on SC-CA1 synapses. Noradrenaline (NE) inhibits the SC-CA1 synaptic transmission by 15%, but suppressed the excitatory synaptic transmission at PP-CA1 synapses even more (~50%)

(Otmakhova and Lisman, 1999, 2000, Otmakhova et al., 2005). In particular, the DA and NE effects at PP-CA1 synapses demonstrate an anatomical position-dependent manner in rat hippocampus, with the largest depression of excitatory synaptic transmission observed at the lateral CA1 area, which receives projection from lateral entorhinal cortex, and little effect observed at medial CA1 sites, which receive medial entorhinal cortex afferents (Ito and Schuman, 2012). Furthermore, DA is able to facilitates the activity-driven synaptic plasticity at PP-CA1 synapses in a stimulation frequency- dependent manner (Ito and Schuman, 2007).

### ***1.5: Hebbian synaptic plasticity at PP-CA1 synapses***

PP-CA1 synapses at distal dendrites undergo activity-dependent LTP and LTD. Initial reports failed to elicit LTP at PP-CA1 synapses in the absence of GABA<sub>A</sub> antagonist. However, low frequency stimulation (10Hz) of PP input induces LTD at PP-CA1 synapses with intact inhibitory synaptic transmission (Dvorak-Carbhone and Schuman, 1999). Later, Schuman et al successfully evoked LTP *via* a strong high frequency stimulation protocol (Remondes and Schuman, 2002). Subsequent investigation showed that PP LTP requires the activation of NMDA receptors, voltage sensitive calcium channels, presynaptic GABA<sub>B</sub> receptors and the persistence of such LTP depends on protein synthesis (Remondes and Schuman, 2003).

In addition, an input-pairing stimulation paradigm has been utilized by other researchers to investigate inputs interaction in the CA1 region, such that two pairs of electrodes were employed to stimulate and record PP-CA1 and SC-CA1 responses simultaneously. Theta burst stimulation delivered at the PP input resulted in facilitation of SC LTP/LTD (depending on relative timing of PP and SC input stimulation) if burst stimuli at both inputs are paired in proper time intervals within a theta circle. In turn, simultaneous activation of PP and SC inputs results in large LTP at PP-CA1 synapses. (Remondes and Schuman, 2002, Dudman et al., 2007, Izumi and Zorumski, 2008). Whole cell recordings revealed an all-or-none plateau-like potential elicited stimulation of the PP input, which depends on the Ca<sup>2+</sup> influx through NMDA receptors, group I mGluR (mGluR 1 and mGluR5), putative R-type calcium channels and subsequent calcium release from intracellular calcium reservoirs that relays the Ca<sup>2+</sup> signal to proximal dendrites (Dudman et al., 2007, Takahashi and Magee, 2009). Above threshold stimulation has no effect on the amplitude or the duration of such plateau (Wei et al.,

2001). In conclusion, there is a dialogue between PP and SC inputs within CA1 region, such that SC activity is capable of gating PP post-synaptic activities to soma and enhances the magnitude of PP LTP/LTD, while SC synaptic transmission can also be boosted by PP activities.

It has been shown that f EPSPs induced at PP-CA1 synapses have a higher NMDA/AMPA receptor component ratio than that induced at SC-CA1 synapses (Otmakhova et al., 2002). Moreover, emerging evidence has shown that the PP and SC inputs activate distinct subtypes of NMDA receptors: GluN2B subunits make a greater contribution to the NMDA component of EPSCs at SC-CA1 synapses (Arrigoni and Greene, 2004). On the other hand, leptin-driven synaptic plasticity in CA1 shows selectivity of NMDA receptor subunits, as leptin induces a long-term or transient depression at SC-CA1 synapses that depend on GluN2B subunit-containing NMDA receptors in young hippocampus, whereas leptin induces long-term potentiation at SC-CA1 synapses in adult hippocampus that depends on GluN2A subunit-containing NMDA receptors (Moult and Harvey, 2011). Thus, it is possible that leptin regulates the PP-CA1 synaptic transmission in a novel way since a differential distribution of NMDA subunits was discovered at distal dendrites of CA1 pyramidal neurons. Here we have used standard extracellular recordings to examine the effects of leptin on the excitatory synaptic transmission at PP-CA1 synapses and the role that GluN2 subunits play in such effect.

## **HYPOTHESIS**

I have summarised how leptin regulates the synaptic plasticity of SC-CA1 synapses in a NMDA subunits and age dependent manner with leptin-induced transient or LTD in young animals dependent on the activation of GluN2B-containing NMDA receptors, whereas leptin-induced LTP at SC-CA1 synapses requires the activation of GluN2A-containing NMDA receptors. On the other hand, GluN2B subunits make greater contribution to the NMDA component of SC synaptic transmission in adult hippocampus. However, the role of leptin in regulating the PP-CA1 synapses is unknown. Also, the distribution of GluN2 subunits at early developmental stages hasn't been elucidated.

## **AIMS**

The aims of this project were as follows:

1. To examine the effects of leptin on synaptic transmission in the distal dendritic region of hippocampal CA1 pyramidal neurons in response to activation of the perforant path.
2. To deepen our understanding about the relationship between GluN2 subunits and the polarity of leptin-induced synaptic plasticity.
3. To compare the cellular expression mechanisms for classical LTP/LTD and the effects of leptin at PP-CA1 synapses.

## **Chapter Two**

### **Materials and Methods**

## 2.1: Materials

### 2.1.1: Reagents

*Table 2.1: Reagents for ACSF*

Compound	Source	Final Concentration (mM)
sodium chloride (NaCl)	Fisher Scientific UK Ltd	124
Potassium chloride (KCl)	Sigma Aldrich Company Ltd	3
sodium bicarbonate (NaHCO <sub>3</sub> )	Sigma Aldrich Company Ltd	26
Sodium phosphate monobasic monohydrate (NaH <sub>2</sub> PO <sub>4</sub> )	Sigma Aldrich Company Ltd	1.25
Magnesium sulphate (MgSO <sub>4</sub> )	Fisher Scientific UK Ltd	1
Calcium chloride (CaCl <sub>2</sub> )	Ascent Scientific	2
D-(+)-Glucose	Sigma Aldrich Company Ltd	10

*Table 2.2: Reagents for Slicing Solution*

Compound	Source	final concentration (mM)
sodium chloride (NaCl)	Fisher Scientific UK Ltd	87
Potassium chloride (KCl)	Sigma Aldrich Company Ltd	2.5

sodium bicarbonate ( $\text{NaHCO}_3$ )	Sigma Aldrich Company Ltd	26
Sodium phosphate monobasic monohydrate ( $\text{NaH}_2\text{PO}_4$ )	Sigma Aldrich Company Ltd	1.25
Magnesium sulphate ( $\text{MgSO}_4$ )	Fisher Scientific UK Ltd	7
Calcium chloride ( $\text{CaCl}_2$ )	Ascent Scientific	0.5
D-(+)-Glucose	Sigma Aldrich Company Ltd	10
Sucrose	Sigma Aldrich Company Ltd	75

**Table 2.3: Pharmacological Tools**

<b>Compound</b>	<b>Source</b>	<b>Vehicle</b>	<b>Stock concentration</b>
Recombinant Human Leptin/OB	R&D Systems	Tris-HCl	50 $\mu\text{M}$
D-AP5	Ascent Scientific	dH <sub>2</sub> O	50mM
Dopamine	Sigma Aldrich Co. Ltd	dH <sub>2</sub> O	10mM
Ro 25-6981 hydrochloride	Sigma Aldrich Co. Ltd	DMSO	10mM
Ifenprodil Tartrate	Ascent Scientific	dH <sub>2</sub> O	3mM

### 2.1.2: Instruments

**Table 2.4: Instruments**



<b>Name</b>	<b>Model</b>	<b>Manufacturer</b>
Micropipette Holder Controller	MP-225	Sutter Instrument Co.
Two Channel Digital Real-Time Oscilloscope	TDS-360	Tektronix
Digital Analogue Converter	1200 series interface	Axon Instruments
Integrating Patch Clamp Amplifier	Axopatch 200B	Axon Instruments
Constant Voltage Isolated Stimulator	DS2A-Mk.II	Digitimer Ltd.
Peristaltic Pump	101U/R	Watson-Marlow Limited
Flaming/Brown Micropipette Puller	P-97	Sutter Instrument Co.
Vibratome	7000 smz	Camden Instruments

## **2.2: Methods**

### **2.2.1: *Animal care***

Sprague-Dawley rats were housed under a 12 hour light/dark cycle and were fed with food and water *ad libitum*. Male rats were mated with female litter mates (3-4 animals per cage). Efforts were made to reduce the number of animals used and minimise their suffering.

### **2.2.2: *Hippocampal slice preparation***

Either male or female 14-23 days old Sprague-Dawley rats were sacrificed by cervical dislocation according to UK Scientific Procedures Act, 1986. The cerebellum and

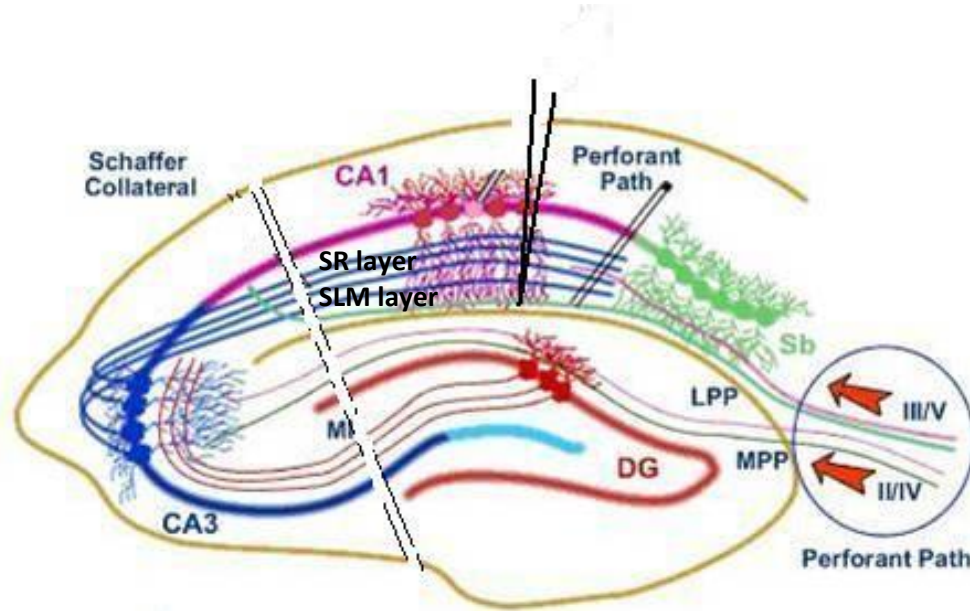
olfactory bulb were removed quickly and the remaining brain was cut into two hemispheres through the inter-hemispheric fissure. The hemispheres were then placed in ice cold high-sucrose artificial cerebral spinal fluid (slicing solution, recipe see above) perfused with 95% O<sub>2</sub>/ 5% CO<sub>2</sub>, pH7.4. Parasagittal slices from the dorsal hippocampus were cut to a thickness of 350 µm using a Vibratome tissue slicer (Campden Instruments Ltd., Loughborough, UK). A cut was made between CA1 and CA2 region as shown on Figure 2.1 to prevent activation of the axons within DG. Slices were incubated in a holding chamber containing oxygenated artificial cerebral spinal fluid (ACSF, recipe see above) at room temperature for at least 1 hour before recording.

### 2.2.3: *Electrophysiology*

Recovered slices were transferred to a recording chamber perfused with ACSF at a flow rate of ~2.5 ml/min. and 95% O<sub>2</sub>/ 5% CO<sub>2</sub>. Experiments were performed at room temperature (~24 °C). Extracellular recordings of field excitatory postsynaptic potentials (fEPSPs) were made from the *stratum lacunosum-moleculare* (SLM) layer of hippocampal CA1 region. In one set of experiments, the Schaffer collateral (SC)-commissural pathway was stimulated to observe the effect of leptin at SC-CA1 synapses. Submerged recording chamber was used for all of our electrophysiological experiments. The recording electrodes were made of glass microelectrodes filled with ACSF (resistance = ~1MΩ). Field responses were evoked by stimulation of the perforant path to CA1 using a handmade bi-polar electrode (twisted nickel-chromium wire) at a frequency of 0.0333 Hz using a stimulus intensity that evoked 50% of the maximum fEPSP initial slope (mV/ms). The distance between the tips of two electrodes was approximately 100µm. Recordings were made using an amplifier (Molecular Devices, Sunnyvale, CA, USA) and the data were low pass filtered at 2 kHz and digitally sampled at 10 kHz. Electronic signals were recorded online and reanalysed offline using winLTP software (Courtesy of Dr Bill Anderson, University of Bristol, UK). Both the amplitudes and the initial slopes of the field post-synaptic responses were recorded, and only the slopes were used for data analysis.

The SLM layer can be subdivided to two layers: the proximal layer that close to SR and the distal layer which adjacent to the fissure between hippocampus formation and DG. We placed the electrodes on the distal layer from where we can acquire larger response. Furthermore, inputs from medial entorhinal cortex (MEC) project to the medial part of

*SLM* close to CA2, while lateral entorhinal cortex (LEC) form synapses on the lateral half of *SLM* close to subiculum. Unless mentioned specifically in the results part, electrodes were placed on the lateral part of the *SLM*.



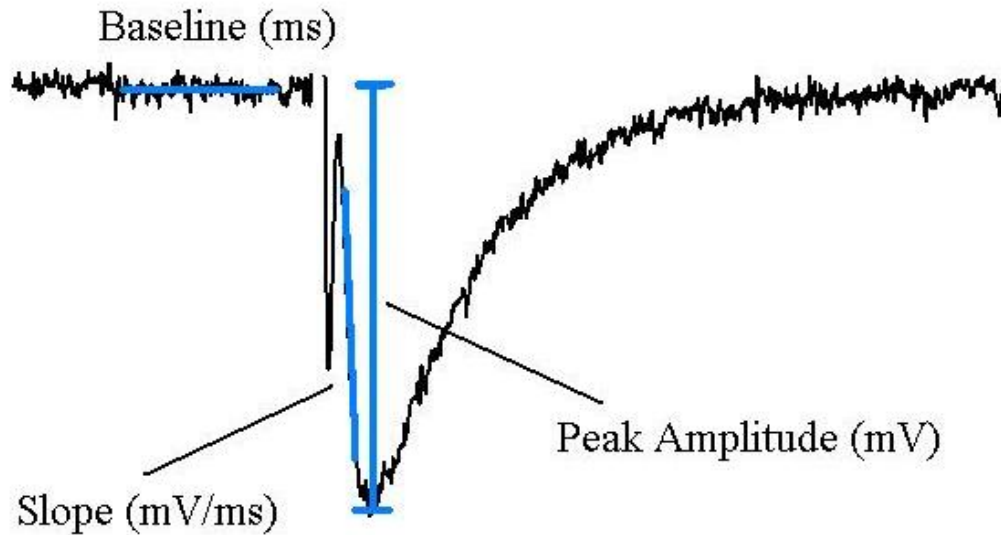
**Figure 2.1: Electrode positions for the perforant path to hippocampal CA1 recording configuration.**

(Adapted from <http://www.bris.ac.uk/anatomy/research/staff/bashir.html>) A cut was made to isolate the PP-CA1 inputs. Parallel lines show the position of the cut. The perforant path fibres from layer III of entorhinal cortex that project to CA1 region were coloured light green. Electrodes were positioned on lateral *SLM* layer.

Water soluble drug stock solutions were made up in sterilized water, water insoluble drugs solutions were made up in DMSO. Drug solutions were dissolved in ACSF and oxygenated before perfusion. All the experiments were performed with normal  $Mg^{2+}/Ca^{2+}$  ratio and intact inhibitory synaptic transmission. Stable baseline recordings of at least 20 min were obtained before the perfusion of drugs.

The high frequency stimulation (HFS) protocol for inducing long-term potentiation (LTP) was 100 pulses at 100Hz (100Hz for 1 second). The protocols were kept the same between control and drug treatment experiments. For paired-pulse facilitation

experiments, two successive identical stimuli were elicited with 50ms interval at a frequency of 0.0333 Hz throughout the experiment. The paired-pulse facilitation ratio (PPR) was calculated as the slope of the second response divided by the slope of the first one.



**Figure 2.2: An example trace of recorded extracellular field excitatory post-synaptic potential (fEPSP) in hippocampal CA1 area.** The fEPSP is generated from simultaneous depolarisation of the apical dendritic area of a population of CA1 pyramidal neurons. The peak amplitude of the fEPSP and the initial slope of the fEPSP are measured. However, the peak amplitude of the fEPSP can be contaminated with population spikes or population inhibitory post-synaptic potentials (pIPSPs). Therefore, the initial slope of the response is used for analysis as a more accurate representation of synaptic transmission.

#### 2.2.4: Data Analysis

Data was reanalysed using winLTP (Anderson and Collingridge, 2001), then imported to Sigmaplot 11.0 (Systat Software Inc.). The slope values of an individual experiment were normalized to the mean of the 20 min baseline slope values using the equation:  $\text{normalized slope values} = (\text{raw data} / \text{mean of baseline slope values}) * 100$ . The magnitude of potentiation or depression was expressed as the percentage of the normalized baseline fEPSP slope. Normalized slope values at the same time points obtained from the same drug treatment paradigm were averaged. Then, 10 successive averaged slope values were chosen from the baseline period and 10 successive averaged

slope values, which represent the maximum effect of drug treatment or maximum potentiation induced by HFS, were chosen from the washout period. One-way ANOVA followed by Holm-Sidak test were performed to measure the difference between baseline values and maximum potentiation values. For the paired-pulse experiments, the significance level of PPR change throughout time was measured by paired t test.

The magnitude of drug effects, long-term potentiation and depression was calculated 20-30 min after the application of leptin or 30-40 min after HFS and expressed as percentage  $\pm$  SEM.  $P < 0.05$  was considered as significant. The n value represents the number of animals used for each set of experiments.

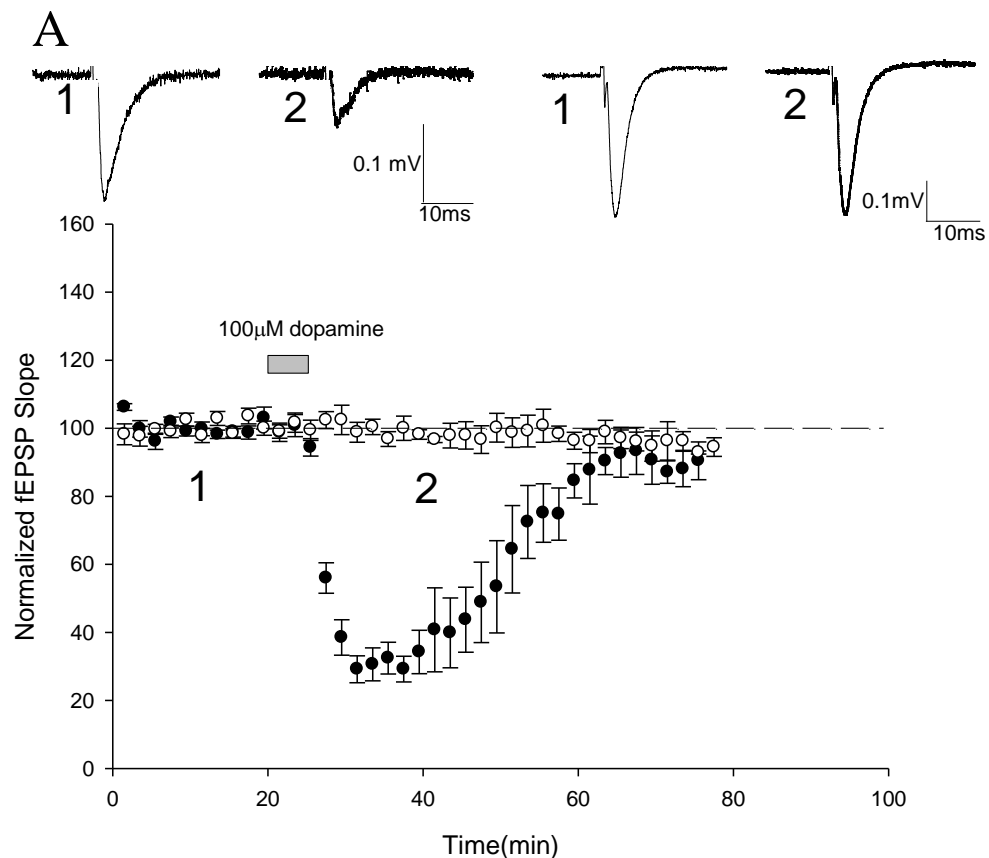
## **Chapter Three**

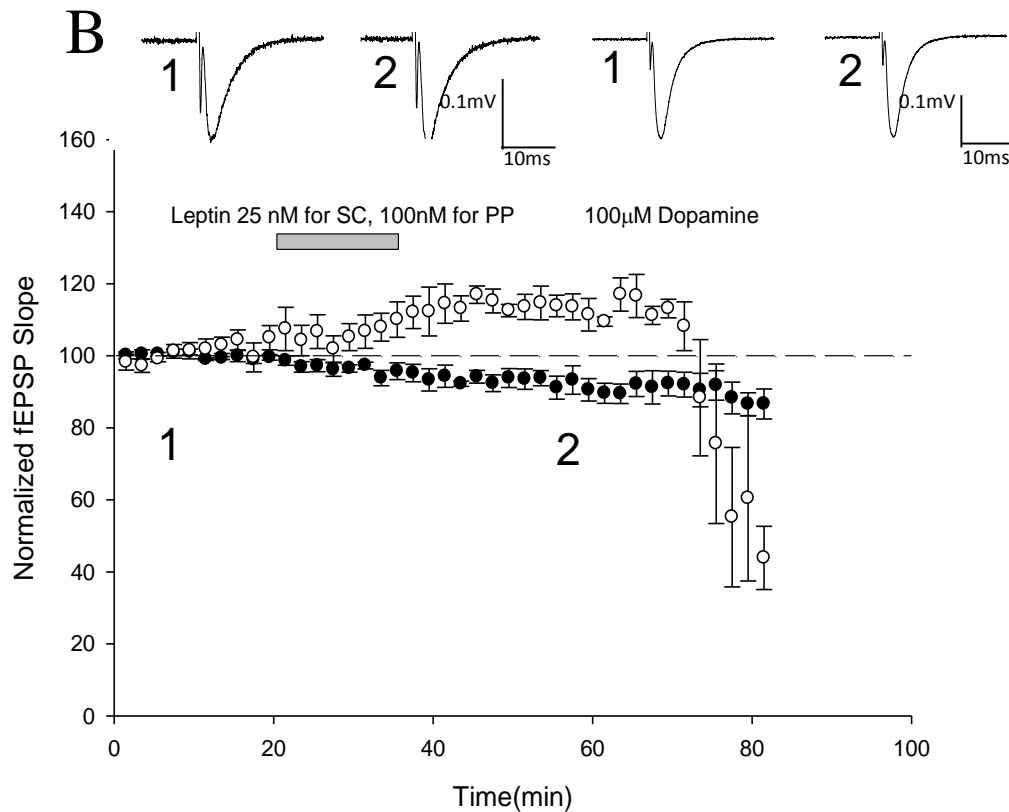
### **Results**

### 3.1: *Leptin induces a long lasting potentiation at PP-CA1 synaptic transmission*

#### 3.1.1 *Isolation of PP-CA1 input*

To isolate PP inputs, previous extracellular studies on PP-CA1 have utilized two pairs of electrodes to record SC and PP simultaneously (Dvorak-Carbone and Schuman, 1999, Otmakhova and Lisman, 1999, Remondes and Schuman, 2002). Activation of SC pathway elicits a positive potential on *SLM* and a negative potential on *SR*. Conversely, stimulation of PP input results in a positive potential on *SR* and negative potential on *SLM*. Here we employed a pharmacological strategy to identify the PP input. It has been shown that the application of high concentration DA (100 $\mu$ M) to PP-CA1 synapses depresses the fEPSP to  $45 \pm 2\%$  of the baseline ( $P < 0.001$ ), whereas 100 $\mu$ M DA shows little effect on SC-CA1 synapses (Otmakhova and Lisman, 1999). In accordance with Otmakhova's work, application of DA for 5 min rapidly depressed the excitatory synaptic transmission at PP-CA1 synapses to  $31 \pm 1.4\%$  ( $n=3$ ,  $P < 0.001$ ) of the baseline after 10 min of washout. On the contrary, no significant effect of DA was observed at SC-CA1 synapses ( $98 \pm 0.8\%$ ,  $P < 0.05$   $n=3$ ,  $P < 0.05$ ), 25-30 min of the washout of leptin was considered as baseline) (see Figure 3.1.1). Thus, we applied 100  $\mu$ M DA at the end of each experiment to verify recording from PP-CA1 synapses.





**Figure 3.1.1 DA rapidly depresses synaptic transmission at PP but not SC input into the CA1 region.**

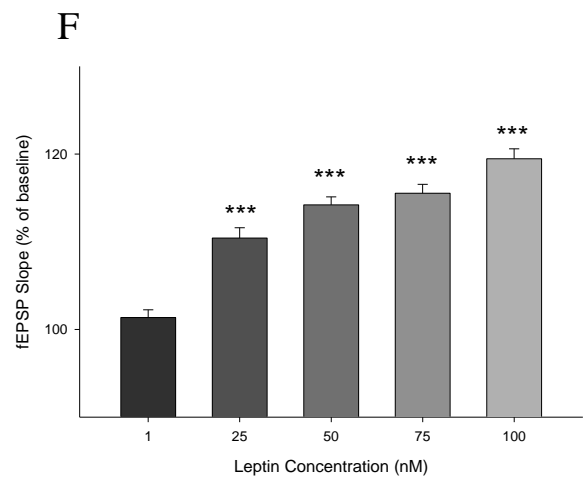
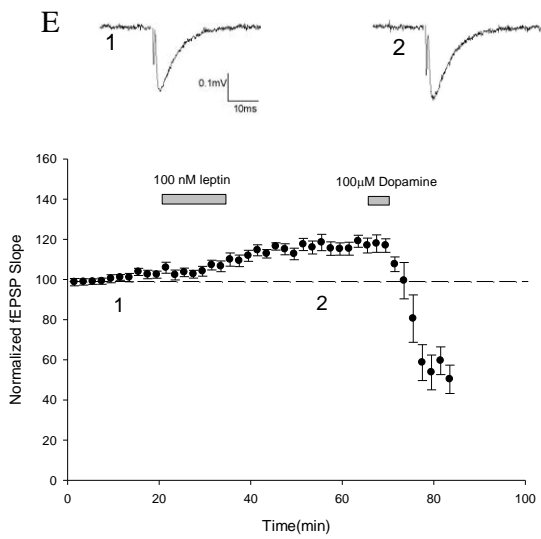
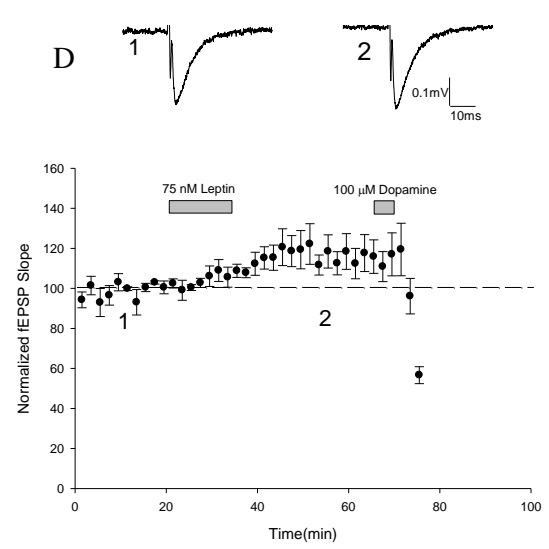
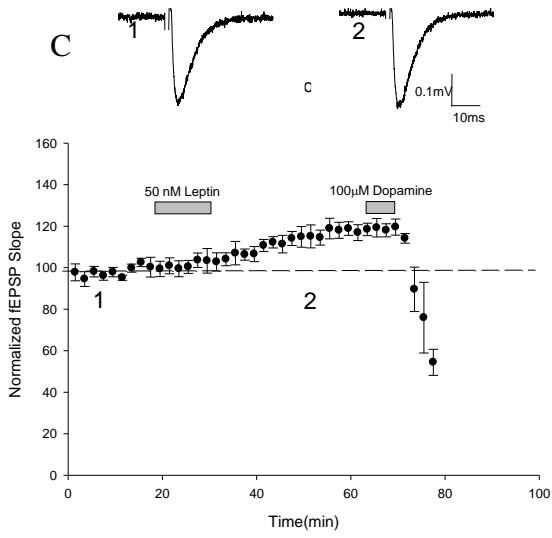
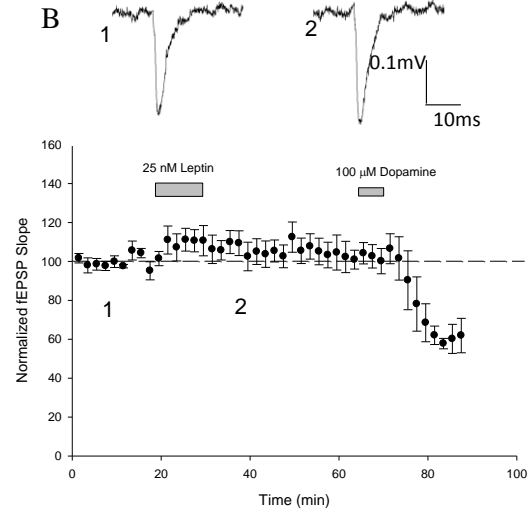
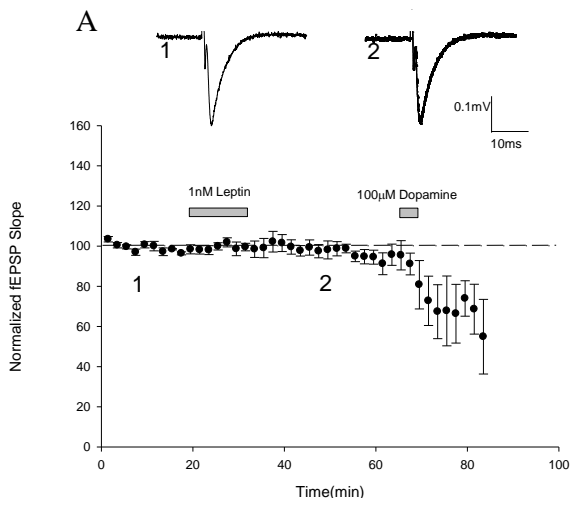
A: Pooled and normalized data shows the effects of 5 min DA application on PP and SC synaptic transmission. Excitatory synaptic transmission at PP-CA1 synapses decreased to  $31 \pm 1.4\%$  of the baseline 10 min after washout of DA (filled circle). But little effect ( $98 \pm 0.8\%$ ) at SC-CA1 synapses can be seen after the washout of DA (open circle). Top, example traces are shown for the time points indicated on the plot. The two traces on the left shows the fEPSP at PP-CA1 synapses, the two traces on the right shows the fEPSP at SC-CA1 synapses. The “1” and “2” on the plot show the position where statistical analysis and example traces were sampled.

B: Pooled and normalized data shows the effect of 5 min DA application at PP and SC synapses following leptin addition. 25 nM leptin was applied to SC, and 100 nM leptin was applied to PP. Hollow dots represent fEPSPs at PP, solid dots represent fEPSPs at SC. The “1” and “2” on the plot show the position where statistics data was sampled. The two traces on the left shows the fEPSP at PP-CA1 synapses, the two traces on the right shows the fEPSP at SC-CA1 synapses. In this and subsequent figures, each synaptic trace is the average of five consecutive responses. Data points represent mean  $\pm$  SEM. Grey bars show the duration of drug treatment.



### 3.1.2: *Leptin effects at PP-CA1 synapses are concentration-dependent*

It has been demonstrated that leptin elicits *de novo* long-term depression (LTD) of excitatory synaptic transmission at SC-CA1 synapses under conditions of enhanced excitability in juvenile rats (Durakoglugil et al., 2005), whereas leptin induces a transient synaptic depression in the presence of picrotoxin in P11-18 rats (Moult and Harvey, 2011). To examine the effects of leptin at PP-CA1 synapses, we applied a range of leptin concentrations for 15 min in the presence of normal ACSF. Application of 1 nM leptin had no effect on the excitatory synaptic transmission ( $101 \pm 0.9\%$ ,  $n=5$ ,  $P>0.05$ ). Application of 25nM leptin evoked a moderate potentiation ( $110 \pm 0.9\%$  of baseline,  $n=4$ ,  $P<0.001$ ) within 10 min of washout, but this effect was transient as synaptic transmission decreased to baseline ( $102 \pm 0.6\%$  of baseline,  $n=4$ ,  $P>0.05$ ) 30 min following washout of leptin. However exposure to 50-100nM leptin clearly enhanced synaptic transmission during the 15 min application and the effect was maintained for 30 min (for 50nM leptin:  $118 \pm 0.5\%$  of baseline,  $n=6$ ,  $P<0.001$ ; for 75nM leptin:  $118\% \pm 2.2\%$  of baseline,  $n=4$ ,  $P<0.001$ ; for 100nM leptin:  $119 \pm 1.1\%$  of baseline,  $n=16$ ,  $P<0.001$ ; see Figure 3.2). In conclusion, application of high concentrations of leptin induced a novel form of long-term potentiation on PP-CA1 synaptic transmission in normal ACSF. Thus, 100nM leptin was used for subsequent experiments.

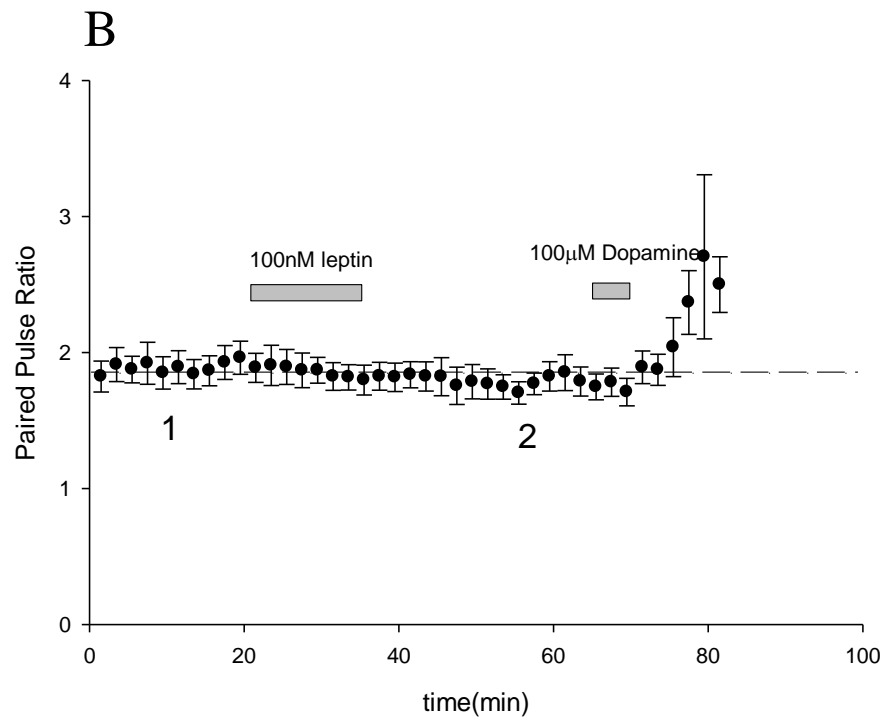
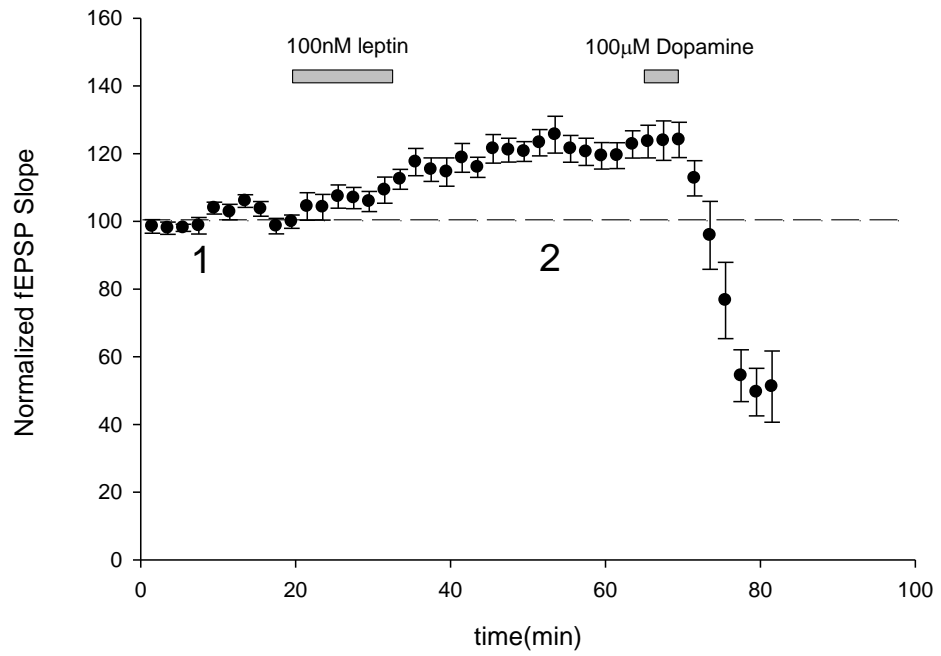


**Figure 3.1.2 The effects of leptin at PP-CA1 synapses are concentration - dependent.**

A-E: Pooled and normalized data shows that application of low concentration of leptin (1nM) had little effect on synaptic transmission at PP-CA1 synapses. However application of higher concentration of leptin (25-100nM) resulted in concentration-dependent increase in the magnitude of LTP. “1” and “2” on the plot show the position where statistical analysis and example traces were sampled. Top, example traces with scale bar are shown for the time points indicated. F: Histogram of pooled data (mean  $\pm$  SEM) illustrating the maximal increase in synaptic transmission induced by the various concentrations of leptin. Significance in one-way ANOVA: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

**3.2: *Leptin-induced LTP at PP-CA1 synapses has a post-synaptic expressing locus***

It has been shown previously that leptin receptors are expressed at both presynaptic and postsynaptic sites in hippocampal neuronal cultures (Shanley et al., 2002). But the locus of leptin-induced potentiation at PP-CA1 synapses is unknown. To examine the locus, we utilized the paired-pulse facilitation protocol as mentioned in methods. Under conditions that leptin (100nM) potentiated synaptic transmission at PP-CA1 synapses to  $122 \pm 1.3\%$  of baseline ( $n=9$ ,  $P < 0.001$ , see Figure 3.2A), no significant change in PPR was detected throughout the experiment ( $n=9$ ,  $P > 0.05$ , paired t test, see Figure 3.2B). As a control, we also examined the effects of DA on PPR on PP-CA1 synaptic transmission. Application of DA (100 $\mu$ M 5min) at the end of the experiment not only reduced synaptic transmission, but also dramatically augmented the PPR from  $1.82 \pm 0.02$  to  $2.52 \pm 0.13$  ( $n=9$ ,  $P < 0.001$  see Figure 3.2B), which is in agreement with studies by (Otmakhova and Lisman, 1999). Thus, our data indicates that leptin-induced LTP at PP-CA1 synapses has a postsynaptic locus of expression.

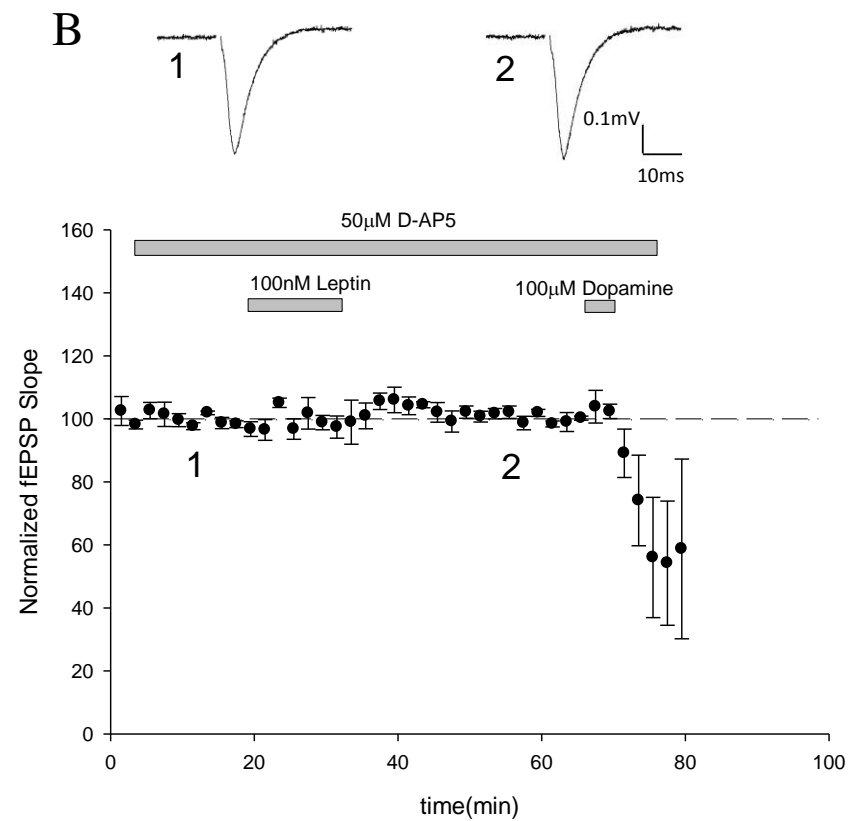
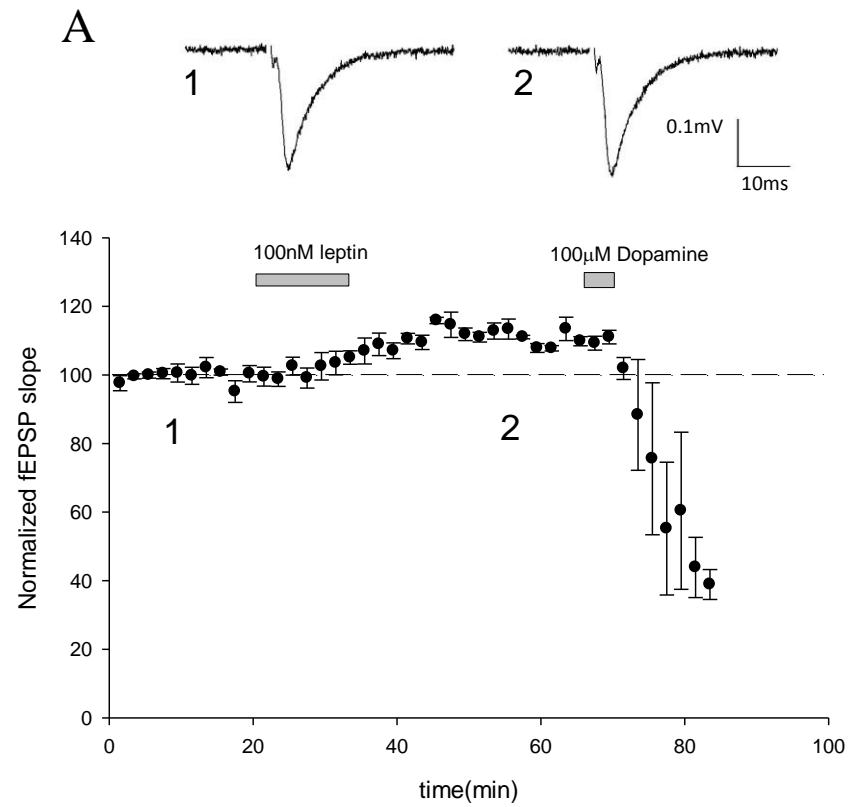


**Figure 3.2: Leptin-induced LTP at PP-CA1 synapses is expressed post-synaptically.**

A: Pooled and normalized data showing that in control slices application of leptin (100nM) rapidly increased synaptic transmission. Top, example traces with scale bar are shown for the time points indicated. B: Plot of the mean paired pulse ratio (50 ms interval) against time for the experiments depicted in A. Leptin had no significant effect on PPF ratio. “1” and “2” on the plot show the position where statistical analysis and example traces were sampled.

***3.3: Leptin-induced LTP at PP-CA1 synapses is dependent on NMDA receptor activation***

Previous studies have shown that in the presence of  $Mg^{2+}$  free ACSF, leptin induces a novel form of LTD at SC-CA1 synapses in juvenile animals, which requires activation of NMDA receptors (Durakoglugil et al., 2005). The ability of leptin to induce LTP at adult SC-CA1 synapses also requires NMDA receptor activation (Moult et al., 2010). It is also known that higher NMDA receptor to AMPA receptor ratio occurs on CA1 pyramidal neurons in *SLM* region (Bittner et al., 2012). Thus, in order to examine the role of NMDA receptors in leptin-induced LTP at PP-CA1 synapses, the NMDA receptor antagonist D-AP5 (50 $\mu$ M) was perfused in the presence of leptin in normal ACSF. In control slices, LTP was induced following exposure to leptin ( $114 \pm 1.0\%$  of baseline,  $n=4$ ,  $P<0.001$ , see Figure 3.3A), whereas in interleaved slices, the ability of leptin to enhance PP-CA1 synaptic transmission was significantly inhibited after 30 min treatment with D-AP5 ( $100 \pm 1.2\%$  of baseline,  $n=4$ ,  $P>0.05$ , see Figure 3.3B). Thus, NMDA receptor activation is involved in leptin-induced LTP at PP-CA1 synapses.

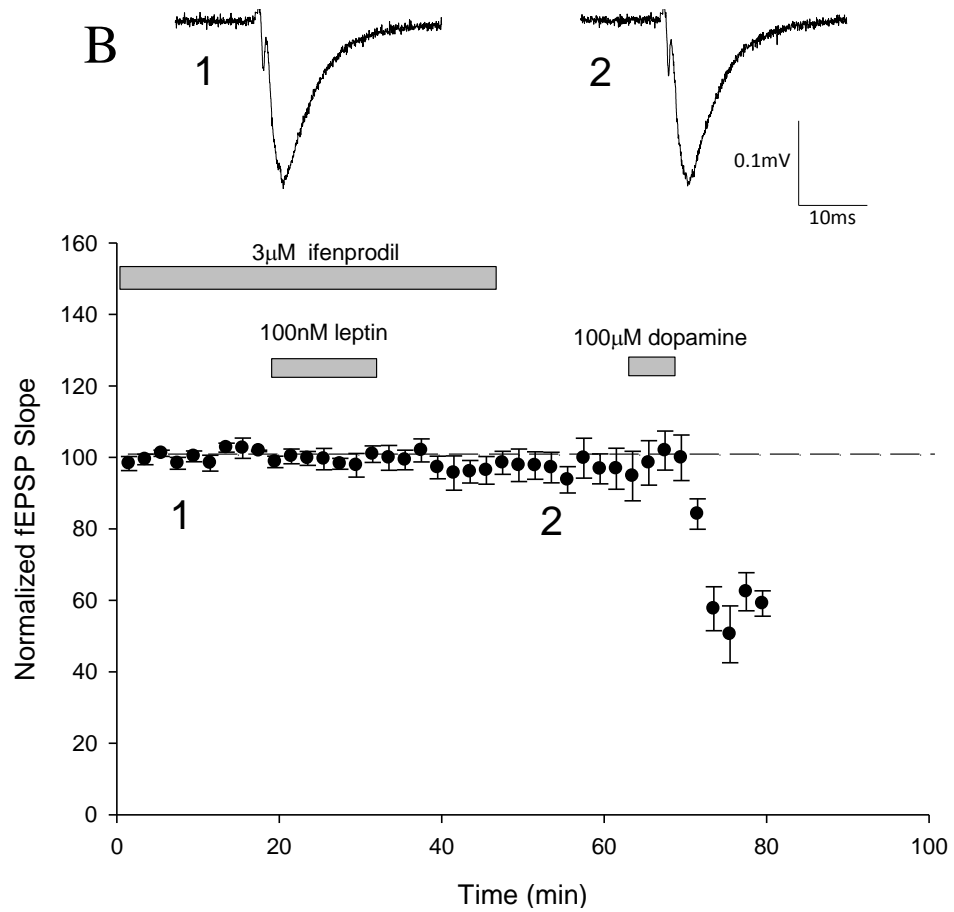
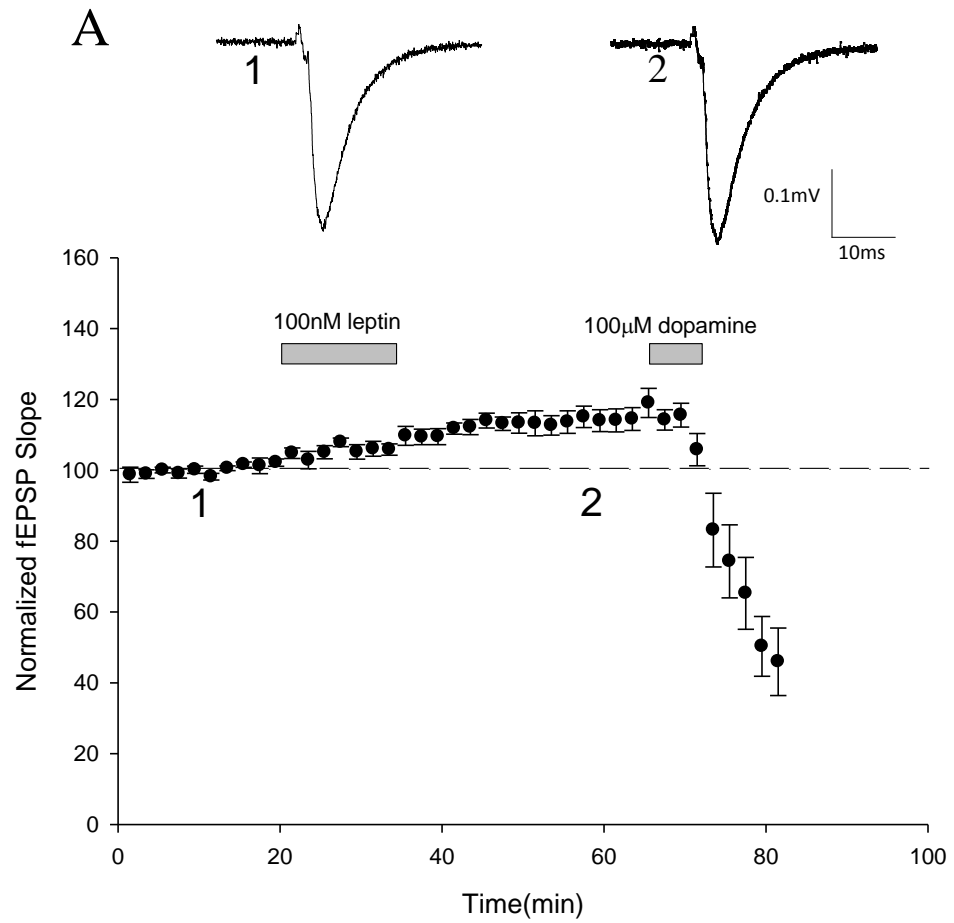


**Figure 3.3: Leptin-induced LTP at PP-CA1 synapses is NMDA receptor dependent**

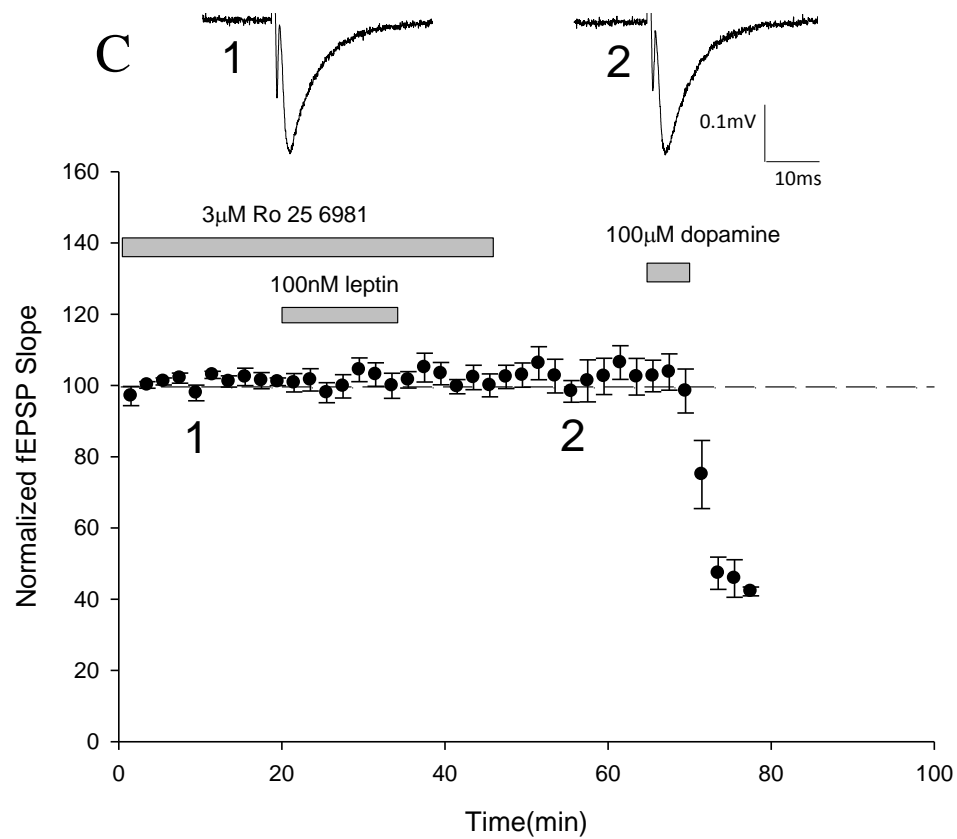
A: Pooled and normalized data showing that application of leptin (100nM) rapidly enhanced the fEPSP slope in control slices. B: The effect of leptin at PP-CA1 synapses is blocked by the competitive NMDA receptor antagonist D-AP5 (50 $\mu$ M). Top, example traces are shown for the time points indicated. “1” and “2” on the plot show the position where subjected to statistical analysis and example traces were

***3.4: The GluN2B subunit of NMDA receptor is required for the leptin-driven LTP at PP-CA1 synapses***

In rat hippocampus, NMDA receptors are composed of at least one GluN1 and two GluN2 (A-D) subunits with an alternative GluN3 subunit. The GluN2 subunits of NMDA receptor undergo switching (GluN2B to GluN2A) in the developing rat brain (Wenzel et al., 1997). Moulton and Harvey 2011 reported that distinct NMDA subunits underline the opposing directions of leptin-induced synaptic plasticity at different stages of postnatal development in rat hippocampus. However the composition of NMDA receptors is not identical in different hippocampal CA1 subregions (Ling et al., 2012). Here, we applied two GluN2B subunit selective antagonists, ifenprodil and Ro-25 6981 to investigate the role of GluN2B in leptin driven LTP at PP-CA1 synapses. In control slices, addition of leptin (100nM) increased the synaptic transmission to  $115 \pm 0.6\%$  of baseline after 30 min washout ( $n=9$ ,  $P<0.001$ , see Figure 3.4A). In contrast, leptin failed to alter synaptic transmission in the presence of ifenprodil ( $99 \pm 0.6\%$  of baseline,  $n=5$ ,  $P>0.05$ , see Figure 3.4B) or Ro-25 6981 ( $101 \pm 0.8\%$  of baseline,  $n=4$ ,  $P>0.05$ , see Figure 3.4C). Together, these data indicates that GluN2B subunit is required for the leptin driven LTP at PP-CA1 synapses in juvenile hippocampus.







**Figure 3.4: Leptin-induced LTP at PP-CA1 synapses requires the activation of GluN2B-containing NMDA receptors**

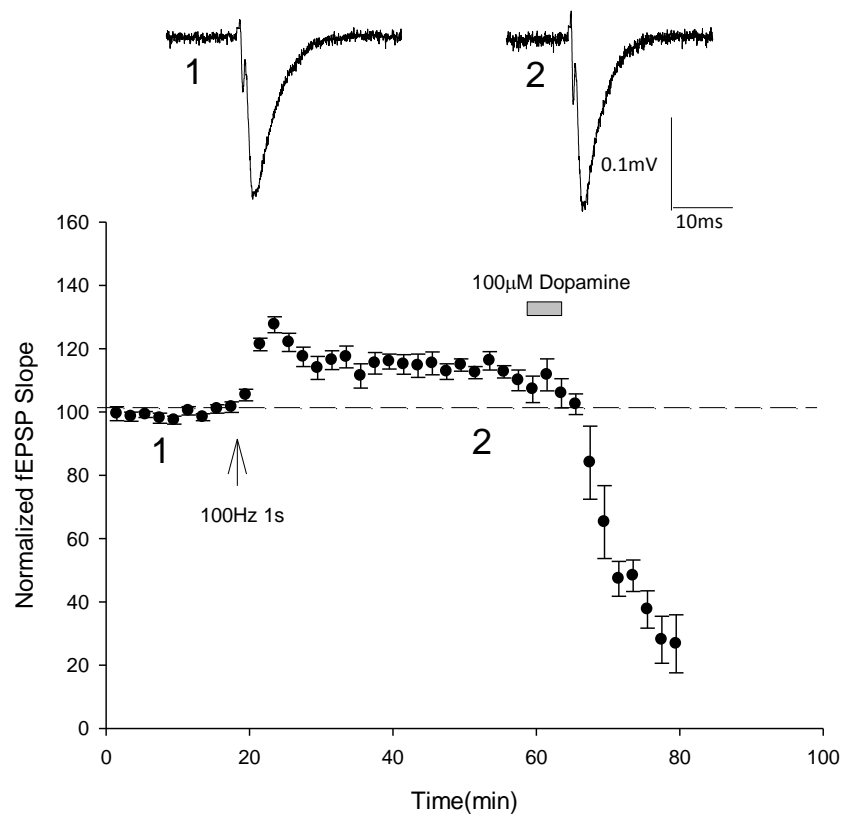
A: Pooled and normalized data demonstrating that application of leptin (100nM) rapidly enhanced the fEPSP slope in control slices. B and C: The effects of leptin at PP-CA1 synapses is blocked by the selective GluN2B subunit antagonist Ro 25-6981 3μM (C) or ifenprodil 3μM (B). Top, example traces are shown for the time points indicated. “1” and “2” on the plot show the position where statistical analysis and example traces were sampled.

### **3.5: Classical Hebbian LTP shares similar mechanism with leptin-induced LTP**

#### **3.5.1: LTP induced by HFS exhibits similar magnitude with leptin-induced LTP at PP-CA1 synapses**

A number of studies have shown that the persistent effects of leptin on the excitatory synaptic transmission share similar mechanisms with classical activity-dependent

synaptic plasticity. In adult rat hippocampus, leptin-induced LTP occludes LTP induced by HFS (Moult and Harvey, 2011). Also, leptin-induced LTD is able to occlude classic Hebbian LTD and *vice versa* (Durakoglugil et al., 2005, Moult and Harvey, 2011). Furthermore, Leptin facilitates the transformation of STP (short term potentiation) into LTP (Shanley et al., 2001) and can reverse established LTP (Shanley et al., 2002). In order to establish if leptin-induced LTP at PP-CA1 synapses shares similar mechanism with classical LTP, we first examined the properties of classical LTP at PP-CA1 synapses. Various protocols were utilized to evoke LTP at these synapses. A relatively weak paradigm (100Hz tetanisation for 1s) was proved most effective in slices from juvenile rats (P14-P20). In accordance with Ito and Schuman, (2012), the magnitude of synaptic transmission peaked within 3-5 min after HFS and then plateaued at the potentiated level for the remainder of the experiment (see Figure 3.5.1). Indeed, at a period 40 min following HFS, the magnitude of synaptic transmission was enhanced to  $114 \pm 1.0\%$  of baseline ( $n=8$ ,  $P<0.001$ , see Figure 3.5.1), which is similar to the magnitude of leptin-induced LTP ( $119 \pm 1.1\%$  of baseline,  $n=16$ ,  $P<0.001$ ; see Figure 3.2).

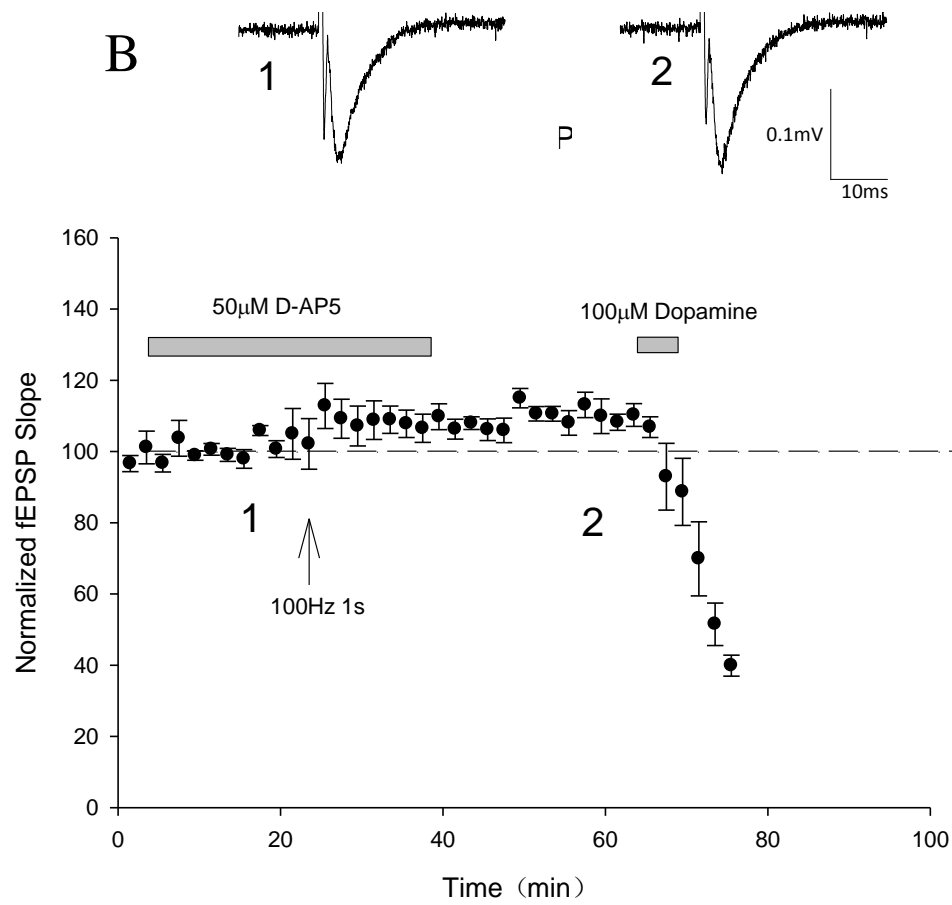
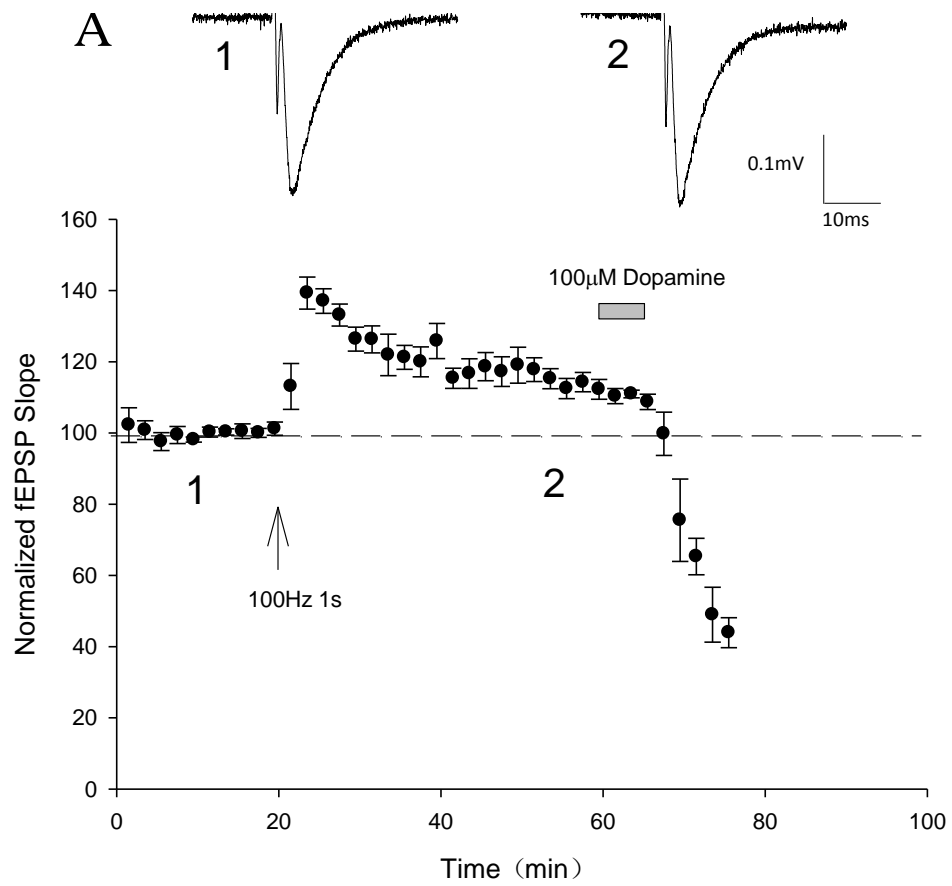


### **Figure 3.5.1: High frequency stimulation induces LTP at PP-CA1 synapses**

Pooled and normalized data demonstrating that HFS (100Hz 1s) stimulation induced long-term potentiation of the PP-CA1 excitatory synaptic transmission. A small potentiation was observed immediately after HFS followed by a LTP which lasted for at least 40 min. Top, example traces are shown for the time points indicated. “1” and “2” on the plot show the position where statistical analysis and example traces were sampled.

### **3.5.2: *Classical LTP at PP-CA1 synapses depends on NMDA receptor activation***

It has been reported previously that activity-dependent synaptic plasticity at PP-CA1 synapses requires the activation of NMDA receptors (Doller and Weight, 1985, Colbert and Levy, 1992, Remondes and Schuman, 2002). In addition, several types of voltage gated calcium channels are involved in LTP at PP-CA1 synapses (Golding et al., 2002, Remondes and Schuman, 2003, Takahashi and Magee, 2009). To examine the role of NMDA receptors that account for LTP at PP-CA1 synapses in juvenile rat slices, the NMDA receptor antagonist D-AP5 was applied. In control slices, LTP was elicited by HFS ( $118 \pm 1.4\%$ ,  $n=5$ ,  $P<0.001$ , see Figure 3.5.2A). However, in interleaved slices treated with D-AP5 ( $50\mu\text{M}$ ), the magnitude of LTP was reduced to  $111 \pm 1.3\%$  ( $n=5$ ,  $P<0.001$ , see Figure 3.5.2B). Thus, LTP at PP-CA1 synapses induced by HFS partially depends on the activation of NMDA receptors.

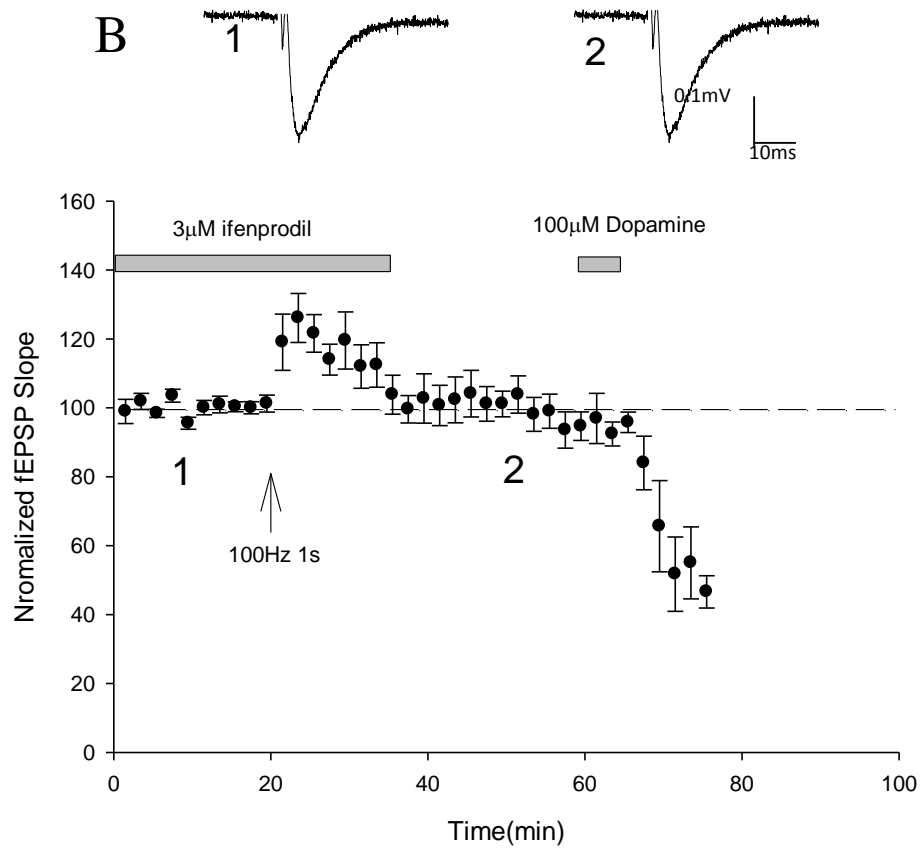
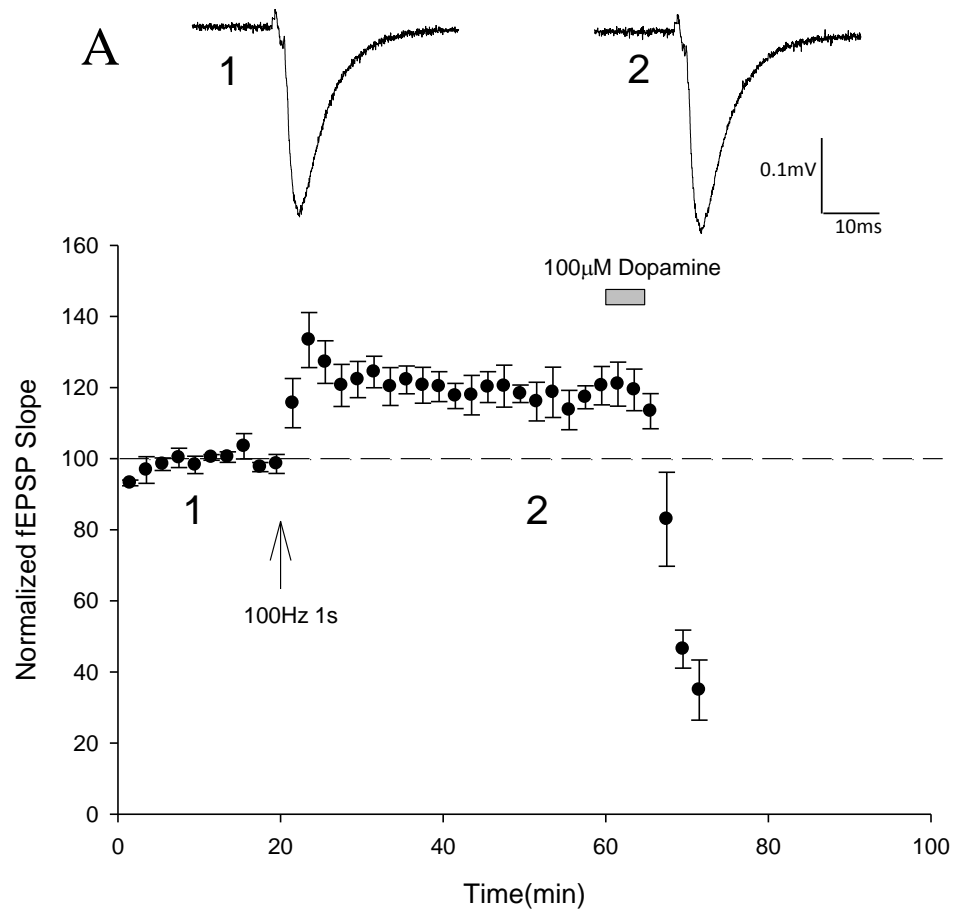


**Figure 3.5.2: Classical LTP at PP-CA1 synapses requires NMDA receptor activation**

A: Pooled and normalized data demonstrating that HFS (100Hz 1s) stimulation induced a long-term potentiation of PP-CA1 excitatory synaptic transmission in control slices. B: The ability of HFS to induce LTP was blocked by 35 min exposure to 50 $\mu$ M D-AP5. Top, example traces are shown for the time points indicated. “1” and “2” on the plot show the positions where statistical analysis and example traces were sampled.

**3.5.3: Classical LTP at PP-CA1 synapses depends on the GluN2B subunits of NMDA receptors**

It has been reported previously that GluN2B subunit is responsible for leptin-driven transient depression of the excitatory synaptic transmission of SC-CA1 synapses in P11-18 rat slices (Moult and Harvey, 2011). In adult rat hippocampus, GluN2B subunits make a larger contribution to the NMDA component of SC-EPSC than that of PP-EPSC (Arrigoni and Greene, 2004). However the GluN2A/GluN2B ratio of NMDA receptor at PP-CA1 synapses is yet to be elucidated. Here, we blocked the GluN2B subunit by selective antagonist ifenprodil in normal ACSF to determine the involvement of GluN2B subunits in PP-LTP in juvenile (P14-18) rat slices. In control slices, HFS induced LTP such that after 40 min, synaptic transmission increased to  $116 \pm 1.0\%$  of baseline, (n=4,  $P < 0.001$ , see Figure 3.5.3A). In interleaved slices treated with 3 $\mu$ M ifenprodil, LTP was inhibited ( $98 \pm 1.2\%$  of baseline, n=4,  $P > 0.05$ , see Figure 3.5.3B) although a small short term potentiation following HFS was still observed. Hence, the PP-LTP evoked by HFS in juvenile (P14-P18) rat depends on GluN2B subunits.

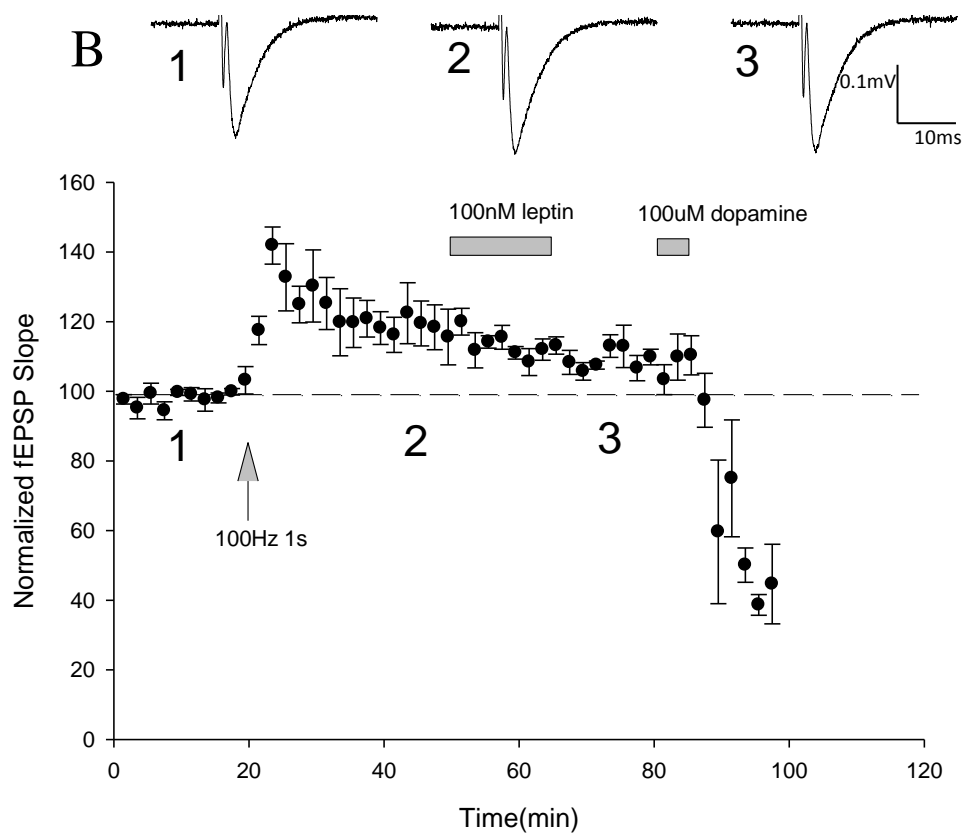
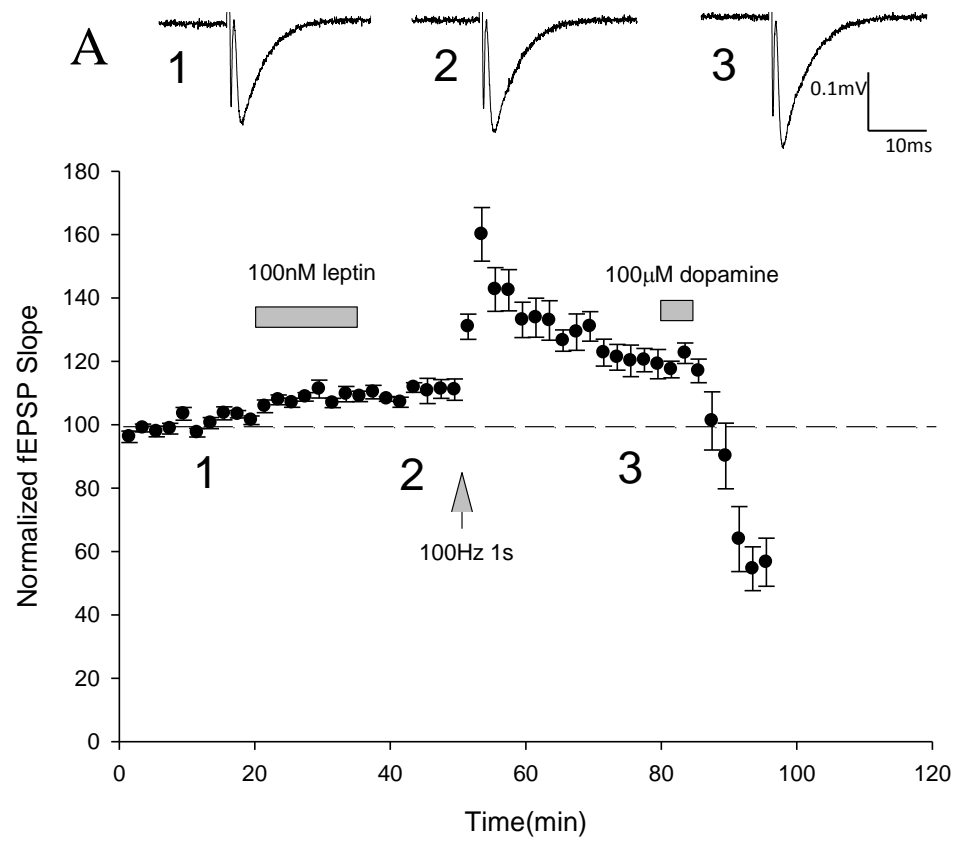


**Figure 3.5.3: Classical LTP at PP-CA1 synapses requires GluN2B receptor activation**

A: Pooled and normalized data demonstrating that HFS (100Hz 1s) induced long-term potentiation at PP-CA1 synapses in control slices. B: HFS induced LTP was blocked by treatment with ifenprodil (3 $\mu$ M). Top, example traces are shown for the time points indicated. “1” and “2” on the plot show the position where statistical analysis and example traces were sampled.

**3.5.4: *Leptin-induced LTP and classical LTP at PP-CA1 synapses partially occlude each other***

Our data has demonstrated that leptin-induced LTP at PP-CA1 synapses share similar phenomena with activity-induced classical LTP at these synapses. To examine further if the two types of synaptic plasticity involve similar mechanisms, we employed occlusion experiments. In the first set of experiments, leptin was initially applied, then HFS was delivered following 15 min washout of the application of leptin. Addition of leptin induced LTP to  $111 \pm 1.4\%$  of baseline, (n=7,  $P < 0.001$  see Figure 3.5.4A). Subsequent HFS resulted in further potentiation of PP-CA1 synaptic transmission ( $120 \pm 1.0\%$  of baseline, n=7,  $P < 0.001$  see Figure 3.5.4A). In the second series of experiments, classical LTP was induced *via* HFS first, then 30 min after the HFS, leptin was applied for 15 min. This time leptin induced LTP was completely occluded by classical HFS-driven LTP, as the synaptic transmission at PP-CA1 synapses showed no further increase during or after leptin treatment. Instead, it decreased from  $119 \pm 1.3\%$  to  $109 \pm 1.1\%$  of baseline. (n=4,  $P < 0.001$  see Figure 3.5.4B). Thus, we conclude that leptin-induced enhancement of synaptic efficacy at PP-CA1 synapses and classical LTP share some similar expression mechanisms, as classical LTP is able to occlude leptin-induced LTP, whereas leptin-induced LTP failed to completely occlude classical LTP.





**Figure 3.5.4: Classical LTP and leptin-induced LTP at PP-CA1 synapses share partial expression mechanism**

A: Pooled and normalized data demonstrating that addition of leptin (100nM 15min) partially occluded HFS (100Hz 1s) induced LTP at PP-CA1 synapses, as the synaptic transmission at PP-CA1 synapses was further potentiated ~10% by the HFS. B: HFS (100Hz 1s) induced LTP completely occluded the effect of leptin at PP-CA1 synapses. Top, example traces are shown for the time points indicated. “1”, “2” and “3” on the plot show the positions where statistical analysis and example traces were sampled.

## **Chapter Four**

### **Discussion**

#### ***4.1: Bi-directional regulation of PP and SC inputs to CA1 by leptin***

Our data demonstrates that leptin induces a novel form of long-term potentiation at PP-CA1 synapses with the synaptic transmission increased to ~20% under conditions with normal inhibitory synaptic transmission in juvenile rat slices. On the contrary, leptin has little effect on basal excitatory synaptic transmission at SC-CA1 synapses with intact inhibitory transmission at the same age (Durakoglugil et al., 2005). However, leptin elicits long-term depression of synaptic transmission at SC-CA1 synapses in the presence of either low  $Mg^{2+}$  containing ACSF or GABA<sub>A</sub> receptor antagonist picrotoxin (see Figure 3.1.1B and Durakoglugil et al., 2005). One possible explanation for the difference of the magnitude and directions of the leptin effects at the two distinct excitatory inputs to the CA1 region is that leptin may differentially regulates inhibitory inputs that control the PP and SC inputs respectively. Mounting evidence showing that in the hippocampal CA1 region, PP and SC inputs onto pyramidal cells are regulated by distinct types of interneurons (Parra et al., 1998, Klausberger and Somogyi, 2008, Kullmann, 2011). (Solovyova et al., 2009) reported that leptin promotes a GABA<sub>A</sub> receptor-mediated rapid and reversible increase of the amplitude of IPSC in hippocampal CA1 pyramidal neurons by activating the SC fibres. After washout of leptin, a long-term depression of IPSC was observed. This study suggests that the effect of leptin on the excitatory synaptic transmission at SC-CA1 synapses may be counteracted by the GABA<sub>A</sub> receptor-mediated feed-forward inhibition. On the other hand, GABA<sub>B</sub> receptor mediated synaptic transmission can also be activated by PP and SC inputs, and GABA<sub>B</sub> receptor is required by both PP-CA1 LTP and SC-CA1 LTP (Davies et al., 1991, Remondes and Schuman, 2003). However, the potential role of leptin on the inhibitory synaptic transmission at PP-CA1 synapses is unclear. Thus, it is possible that the differential regulation of leptin on GABA<sub>A</sub> and/or GABA<sub>B</sub> mediated synaptic transmission activated by PP stimulation may account for the relatively large effect of leptin at the PP excitatory synaptic transmission.

Another possibility that accounts for the different effects of leptin at the two pathways may derive from the cooperative property of NMDA receptors at distal dendrites of CA1 pyramidal neurons. Recent studies have shown that the NMDA receptor to AMPA receptor ratio is higher in distal tuft dendrites of CA1 pyramidal neurons (Bittner et al., 2012), which may results in larger NMDA/AMPA charge ratio of EPSC at PP compared with SC (Otmakhova et al., 2002). Moreover, photostimulation studies

indicate that activated NMDA receptors in a single dendritic spine are able to facilitate the activation of NMDA receptors in adjacent spines within the same dendritic branch and generate dendritic spikes, which results in the cooperative LTP at PP-CA1 synapses (Wei et al., 2001, Golding et al., 2002, Losonczy and Magee, 2006, Harvey et al., 2008). Given that leptin-driven LTP in the hippocampus shares similar mechanism with classical activity-driven LTP (Moult and Harvey, 2011), it is feasible that leptin-driven signalling pathways recruit cooperative activation of more NMDA receptors at distal dendrites and evoke subsequent LTP that exhibits larger effect than SC-CA1 synapses.

In addition, leptin receptor labelling and *in situ* hybridization studies show that leptin receptor and its mRNA are highly concentrated in *SLM* layer of CA1, CA2 CA3 and the molecular layer of DG (Elmqvist et al., 1998, Scott et al., 2009, Patterson et al., 2011), where PP form synapses onto neurons in the hippocampus. More clustered leptin receptors in these regions may enable more NMDA receptors to be activated that eventually lead to larger magnitude of LTP.

#### ***4.2: Presynaptic mechanism of classical LTP at PP-CA1 synapses***

The paired-pulse facilitation experiments show that leptin-induced LTP at PP-CA1 synapses has a post-synaptic locus of expression. Nevertheless, a recent study indicates that certain presynaptic mechanisms are involved in the generation of classical LTP at PP-CA1 synapses (Ahmed and Siegelbaum, 2009). Under basal conditions, the release probability of neurotransmitter from presynaptic terminal at PP-CA1 synapses is lower than that of SC-CA1 synapses due to minor contribution of presynaptically expressed N-type voltage dependent  $\text{Ca}^{2+}$  channels. Application of a 200Hz tetanic stimulation paradigm enhances the contribution of N-type  $\text{Ca}^{2+}$  channels and the augments the release efficacy that account for LTP at PP-CA1 synapses (Ahmed and Siegelbaum, 2009). In addition, Xu et al., 2008 found that leptin-induced depression of the excitatory synaptic transmission in juvenile murines is paralleled by a small alternation of PPR. Thus, we cannot rule out the possibility that a potential presynaptic mechanisms also contribute to leptin-driven LTP at PP-CA1 synapses (Xu et al., 2008).

#### ***4.3: Comparison of leptin effects with monoamine neuromodulators modulation at PP-CA1 synapses***

Our data shows that treatment with leptin induces a long lasting potentiation of the excitatory synaptic transmission at PP-CA1 synapses. In contrast, the three monoamine neuromodulators DA, 5-HT and NE selectively and reversibly depress PP-CA1 synaptic transmission *in vitro* (Otmakhova and Lisman, 1999, 2000, Otmakhova et al., 2005). Indeed, leptin and the three monoamines display a series of distinct characteristics. First of all, although the three monoamines regulate various physiological activities outside CNS, they are synthesised in a small group of specialized neurons in the CNS and diffuse through large areas of the brain *via* long range projections. Whereas leptin is secreted predominately outside CNS and enters the CNS by crossing the brain-blood barrier (Banks et al., 1996) or entering the cerebrospinal fluid (Schwartz et al., 1996a). Secondly, mounting evidence shows that the concentration of DA is dynamically and strictly regulated in the CNS, such that DA concentration stays in nM range in resting animals, and increases dramatically to  $\mu$ M range following exposure of novel stimuli or electronic stimulation of dopaminergic neurons (Garris et al., 1997, Ihalaenen et al., 1999, Chen and Budygin, 2007). *In vitro*, DA, 5-HT and NE induced depressions of synaptic transmission at PP-CA1 synapses show concentration dependent manner (Otmakhova and Lisman, 1999, 2000, Otmakhova et al., 2005), which provides further evidence for the phenomena mentioned above. In contrast, circulating leptin levels exhibit pulsatile rhythm with ~30 pulses of leptin secretion within 24 hours (Licinio et al., 1997, Schubring et al., 1999). Also, leptin level is under control of energy intake in both acute (5~8 hour) and chronic (within 24 hour) manner (Boden et al., 1996, Kolaczynski et al., 1996). Although active concentrations of leptin in the brain are unknown, it is clear that leptin level in the plasma reflects the amount of energy intake, whereas DA concentration hinges on cognitive process in the brain.

Leptin application in hippocampal slices results in a long lasting potentiation of synaptic transmission at PP-CA1 synapses after washout. However, DA, 5-HT and NE application induce reversible depression at PP-CA1 synapses. The oppositional polarities of synaptic plasticity driven by leptin and monoamines may reflect their distinct role in PP associated cognitive function in the juvenile animal. Indeed, local infusion of the non-selective DA agonist apomorphine (10, 15  $\mu$ g), into the CA1 subregion of awake animals produces impairments in working memory and spatial memory tasks (Vago and Kesner, 2008). Conversely, the administration of leptin into the hippocampus promotes the behavioural performance in emotion and spatial memory related tasks (Farr et al.,

2006, Oomura et al., 2006). Hence, it is feasible to conject that leptin functions as a tonic cognitive enhancer that facilitates NMDA receptor-associated learning and memory process in juvenile hippocampus. Given that the magnitude of the leptin effect at PP-CA1 synapses is relatively larger than the effect at SC-CA1 synapses, a major target of leptin modulation in the hippocampal CA1 region might be the sensory information conveyed by the direct EC-CA1 circuit. On the other hand, monoamines selectively inhibit the direct cortical input to influence the information comparison within the CA1 region.

#### ***4.4: The role of GluN2 subunits in NMDA receptor-dependent synaptic plasticity***

In accordance with our previous studies (Shanley et al., 2001, Durakoglugil et al., 2005, Moulton et al., 2009, Moulton and Harvey, 2011), the competitive NMDA receptor antagonist D-AP5 significantly blocked leptin-induced LTP at PP-CA1 synapses, indicating the involvement of NMDA receptors in such a process. Furthermore, the ability of two GluN2B selective antagonists ifenprodil and Ro 25-6981 to completely inhibit the leptin-driven augmentation in excitatory synaptic strength at PP-CA1 synapses in juvenile hippocampus clearly identifies the involvement of GluN2B subunits rather than GluN2A. Nevertheless, the activation of GluN2B-containing NMDA receptors evoked by leptin treatment leads to a transient or long-term depression of SC-CA1 synaptic transmission in juvenile (P11-P18) hippocampus (Durakoglugil et al., 2005, Moulton and Harvey, 2011). Indeed, the contribution of different GluN2 subunits in activity-dependent synaptic plasticity and learning is still a controversial issue. Clayton et al., (2002) utilized antisense oligonucleotides to selectively knock-down GluN2B subunits in the hippocampus of young rats. They found that antisense treatment attenuated NMDA receptor-mediated responses, abolished NMDA-dependent long-term potentiation (LTP), and impaired spatial learning (Clayton et al., 2002). Accordingly, Tang et al., (2010) reported that overexpression of the GluN2B subunit results in enhanced LTP and spatial learning (Tang et al., 2010). In a report by von Engelhardt et al., (2008), genetic ablation of GluN2B subunits spared hippocampal-dependent, hidden-platform water maze performance but impaired a selective, short-term, spatial working memory task (von Engelhardt et al., 2008). Moreover, pharmacological tools have also been used to determine the role of GluN2A and GluN2B subunits in LTP and LTD. Some studies concluded that GluN2A-containing NMDA receptors are responsible for LTP whereas

GluN2B subunit-containing NMDA receptors are required for LTD (Liu et al., 2004, Massey et al., 2004). However, others have reported that both GluN2A and GluN2B subunits are able to induce LTP and/or LTD (Berberich et al., 2005, Toyoda et al., 2005, Zhao et al., 2005). One reason that may account for this discrepancy is the lack of selectivity of the widely-used GluN2A antagonist NVP-AAM077 (Neyton and Paoletti, 2006). In addition, mounting evidence indicates the existence of triheteromeric GluN1/GluN2A/GluN2B receptors in the brain (Cull-Candy and Leszkiewicz, 2004, Rauner and Kohr, 2011), which may complicate this issue.

An alternative hypothesis (Lisman and Raghavachari, 2006, Morishita et al., 2007) is that the amount of calcium influx determines the direction of synaptic plasticity: larger amounts of calcium influx induce LTP whereas less calcium influx is required for LTD. In support of this, the calcium influx activated Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) and associated p38 MAP kinase pathway is involved in NMDA receptor-dependent LTD (Krapivinsky et al., 2003, Li et al., 2006). Whereas, a similar factor Ras-GRF2, which selectively couples with ERK-MAPK pathway may have a role in NMDA receptor-dependent LTP (Li et al., 2006, Jin and Feig, 2010). Our data, together with Moulton and Harvey, (2011) suggest that GluN2B subunit plays a role in both LTP and LTD initiated by leptin receptor activation at different dendritic areas of CA1 pyramidal cells (Moulton and Harvey, 2011), which highlights the pivotal role of GluN2B in hippocampal synaptic plasticity at early developmental stages and provided further evidence for the hypothesis outlined above.

In the hippocampus, there is differential expression of the NMDA receptor subunits at different development stages. Thus, GluN2B subunit expression peaks at early postnatal stages in neonatal animals, whilst GluN2A subunit expression increases gradually during development (Monyer et al., 1994, Sans et al., 2000). A previous study revealed that leptin-driven bi-directional regulation of NMDA receptor-dependent synaptic plasticity at SC-CA1 synapses at different postnatal developmental stages parallels the age-dependent expression of NMDA receptor subunits. In particular, the GluN2B-dependent transient (P11-18) or long-term depression (P5-8) induced by leptin in young hippocampus is mediated by ERK-MAPK signal pathway. In contrast, the GluN2A-dependent LTP in adult hippocampus is regulated by PI 3-Kinase pathway (Moulton and Harvey, 2011). Hence, it appears that GluN2A and GluN2B subunits are coupled to distinct signalling pathways downstream of the leptin receptor. It would be

intriguing to see if similar pathway dependence of leptin-driven synaptic plasticity at PP-CA1 synapse can be observed.

#### ***4.5: Leptin-induced LTP shares similar mechanism with classical LTP at PP-CA1 synapses***

Several lines of evidence have demonstrated that leptin-induced modification of synaptic strength share similar expression mechanism with classical activity-dependent synaptic plasticity in the hippocampus (Shanley et al., 2001, Durakoglugil et al., 2005, Moulton et al., 2010, Moulton and Harvey, 2011). We further investigated the possibility that this also occurs at PP-CA1 synapses by comparing classical LTP with leptin-driven LTP at PP-CA1 synapses. In contrast to previous studies, we elicited classical LTP using a moderate stimulation protocol: (100 Hz tetanic stimulation for 1 s), whereas stronger protocols have to be employed to evoke substantial LTP in adult animals (Remondes and Schuman, 2002, 2003). Such age-dependent characteristics may reflect differential dynamics of NMDA receptor subunits at distinct developmental stages as we described above. In accordance with other studies (Ahmed and Siegelbaum, 2009, Ito and Schuman, 2012), the magnitude of the excitatory synaptic transmission was potentiated rapidly in 3~5 min immediately after the tetanus and attenuated gradually with time. After 40 min washout, synaptic transmission plateaued at ~115% of the baseline, which is similar to the magnitude of leptin-driven LTP at PP-CA1 synapses.

It has been well documented that classical LTP induced at PP-CA1 synapses requires the activation of NMDA receptors and different types of voltage-gated calcium channels (VGCCs) (Golding et al., 2002, Remondes and Schuman, 2003). In our study, HFS induced LTP at PP-CA1 synapses was partially blocked by the NMDA receptor antagonist D-AP5, indicating that additional mechanisms, probably the activation of VGCCs, may be involved in HFS-induced LTP. VGCCs regulate various cellular processes by mediating fast calcium influx into the cell, resulting in transient increases in intracellular calcium concentration (Stratton and Morrow, 1991, Reichling and MacDermott, 1993). Accumulating data has shown that VGCCs also take part in neuronal synaptic plasticity in the amygdala (Bauer et al., 2002), visual cortex (Yoshimura et al., 2008), hippocampus (Pelkey et al., 2006) and cerebellum (Yamashita et al., 2013). In the hippocampal CA1 region, VGCCs activation is typically evoked by 200Hz stimulation to SC-CA1 synapses (Grover and Teyler, 1990). However, 100Hz



stimulation or pairing theta burst stimuli at PP and SC pathways are sufficient in activating VGCCs at distal dendrites of CA1 pyramidal cell (Remondes and Schuman, 2002, Takahashi and Magee, 2009). As the long distance prevents the invasion of back-propagating action potentials into distal dendrites (Jarsky et al., 2005), calcium influx through VGCCs may play an essential role in generating the dendritic calcium spikes that provide sufficient postsynaptic membrane depolarization necessary to initiate PP-LTP.

Our data clearly shows that classical LTP evoked by HFS at PP-CA1 synapses depends on GluN2B subunit-containing NMDA receptors in juvenile hippocampus, implying that similar expression mechanisms underline the two phenomena. In subsequent occlusion experiments, the ability of leptin to induce LTP was completely occluded by previously induced HFS-driven LTP, whereas HFS-driven LTP was only partially occluded by prior application of leptin, as synaptic transmission was further potentiated by 10% after tetanus. A possible explanation is that although NMDA receptors were saturated by prior leptin treatment, VGCCs at PP-CA1 synapses are still able to be activated and thus mediate the small potentiation triggered by HFS. Alternatively, a small number of NMDA receptors were activated by leptin, causing a subsequent residual potentiation driven by HFS that recruits remainder NMDA receptors. Thus, elaborate investigation of signal pathways downstream to leptin receptors that interact with NMDA subunits is required to elucidate this issue.

#### ***4.6: Leptin-induced increase in synaptic strength requires AMPA receptor trafficking***

AMPA receptor trafficking is considered as one of the commonly observed mechanisms that contributes to postsynaptic expression of activity-driven long-term synaptic strengthening (Barry and Ziff, 2002, Bredt and Nicoll, 2003). In the hippocampus, NMDA receptor activation is essential for triggering the insertion of long-tailed GluA1-containing AMPA receptors to synapses (Lissin et al., 1999). Accordingly, leptin-induced LTP and accompanying AMPA receptor trafficking in adult hippocampus also requires the activation of NMDA receptors, as D-AP5 application prevents leptin-driven LTP (Moult et al., 2010). In addition, leptin-evoked increase in surface expression of GluA1 and synaptic density of GluA2-lacking AMPA receptors in adults is mediated by PI 3-Kinase signal pathway (Moult et al., 2010). Our previous

studies show that leptin-activated PI 3-Kinase pathway interacts with GluN2A subunits in adult hippocampus, which lead to long-term enhancement of synaptic transmission (Moult and Harvey, 2011). Whereas in juvenile hippocampus, leptin activates ERK-MAPK pathway, which in turn signals GluN2B subunits that results in long-term depression (Moult and Harvey, 2011). Thus, it is feasible that leptin regulates the excitatory synaptic strength and the associated AMPA receptor trafficking *via* distinct signal pathways upstream of NMDA receptor. Clarification of the signal molecules downstream to leptin receptor could deepen our understanding about the leptin-driven bi-directional regulation of synaptic plasticity in the hippocampus at different development stages.

#### ***4.7: The potential role of leptin in cognitive functions associated with PP-CA1 synaptic transmission and diseases***

*In vivo* recording studies demonstrate that EC and CA1 neurons share many important firing properties in the process of spatial learning (Blair et al., 2008). The direct cortical input is able to generate characteristic spatial learning- associative firing pattern in hippocampal subregions *in vivo* independent of the classical tri-synaptic circuit (Brun et al., 2002), indicating that the PP-CA1 input plays equally pivotal role as the tri-synaptic circuit in the information processing within the hippocampus. As proposed by theoretical models, linear summation between the two pathways may exists within hippocampal CA1 neurons (Cash and Yuste, 1999, Kali and Freund, 2005), such that spatial memory conserved previously in CA3 recurrent network is compared with direct cortical information flow in CA1, which may facilitate the comparison between previous experience and new memory when an animal is exploring a novel environment. Given that NMDA receptors are more abundantly expressed at the PP-CA1 synapses (Bittner et al., 2012), NMDA receptor- dependent long-term synaptic plasticity might be involved in the formation and maintenance of EC-CA1 spatial learning- associative firing pattern, since the firing pattern of single cells can last for days (Brun et al., 2002). Thus, future works of pathway-specific lesion or direct administration of leptin onto CA1 perforant path may deepen our understanding about the role of leptin in regulating the information flow from direct cortical input and shaping the action potential firing.

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss and cognitive dysfunction. Two of the most well characterized

pathological features at cellular level in AD are senile plaques and neurofibrillary tangles (NFT) (Watson and Seiden, 1984). Senile plaques are found extracellularly, which consist of aggregated amyloid- $\beta$  peptides (Glenner and Wong, 1984). NFT represents the intracellular deposits of hyper-phosphorylated tau protein (Grundke-Iqbal et al., 1986). Substantial evidence indicates that leptin can reduce the accumulation of amyloid- $\beta$  by mitigating the synthesis and/or augmenting the clearance and degradation of amyloid- $\beta$  (Fewlass et al., 2004). In the hippocampus, leptin inhibits the detrimental effects of amyloid- $\beta$  (1-42) on LTP and amyloid- $\beta$  (1-42) -mediated facilitation of LTD. Also, leptin prevents the internalization of GluA1 *via* activating PI3-kinase (Doherty et al., 2013). On the other hand, leptin is able to attenuate the GSK3 $\beta$  induced hyper-phosphorylation of tau protein *via* Akt, p38 MAPK and AMPK signal pathways (Greco et al., 2008, Greco et al., 2009, Marwarha et al., 2010). Moreover, leptin promotes neurogenesis and facilitates memory formation that rescues the memory impairment and cognitive dysfunction in AD (Perez-Gonzalez et al., 2011). In addition, spatial memory impairment is one of the landmark symptoms in AD patients (Bublak et al., 2006). It has been proved that PP-CA1 synaptic transmission is involved in hippocampal-dependent spatial memory (Vago and Kesner, 2008). Hence, leptin modulation of synaptic plasticity at PP-CA1 synapses highlights its potential role in rescuing AD-associated spatial memory deficiency.

Neuronal excitotoxicity, which is manifested as dysfunction of glutamatergic synaptic transmission, has been hypothesized as an etiology of AD (Lawlor and Davis, 1992). Targeting the NMDA receptors, memantine was developed as a novel approach to treat AD patients. Memantine is an uncompetitive voltage-dependent NMDA receptor antagonist. By binding to NMDA receptors, memantine inhibits the prolonged  $\text{Ca}^{2+}$  influx through extrasynaptic NMDA receptors and maintain the function of synaptically expressed NMDA receptors (Rogawski and Wenk, 2003). Similarly, leptin regulates hippocampal synaptic plasticity *via* activating NMDA receptors (Shanley et al., 2001). Thus, a further elucidation of leptin modulation of NMDA receptor-dependent synaptic plasticity in AD patients may benefit our understanding towards the pathology of AD and developing new drugs.

## **Chapter Five**

## **Bibliography**

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